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Introducing our new look

The National Research Council Publishing Office is please to launch a new design for the *Canadian Journal of Fisheries and Aquatic Sciences* with the first issue for 1995. Although the primary purpose of the redesign was to improve readability, it also incorporates contemporary typographic standards and takes advantage of the design opportunities available in the new computer typesetting technologies. We hope that our readers and subscribers will be pleased with the clear and dramatic new layout and typographic style.

The Publishing Office wishes to acknowledge the valuable assistance of the Design Office of The University of Toronto Press in creating the new design. The involvement of the staff of the Publishing Office is also acknowledged; their input was critical to developing a workable design.

La Revue arbore un nouveau « look »

Le Bureau des publications du Conseil national de recherches est fier d'inaugurer la nouvelle présentation de le *Journal canadien des sciences halieutiques et aquatiques* avec ce premier numéro de 1995. Bien que la nouvelle mise en page ait pour objectifs principal d'améliorer la lisibilité, celle-ci prévoit une typographie enrichie et mise sur le potentiel des nouvelles technologies de publication assistée par ordinateur. Nous espérons que nos lecteurs et nos abonnés conviendront de la supériorité de la nouvelle mise en page et du nouveau style typographique.

Le Bureau des publications désire souligner l'aide inestimable du Bureau de conception graphique de la University of Toronto Press dans la refonte de notre mise en page. Nous désirons aussi exprimer nos remerciements aux employés du Bureau des publications, dont le rôle a été crucial dans l'élaboration d'une mise en page efficace.

Mrs. Aldyth Holmes

Director / directrice

Selective tidal stream transport of adult sockeye salmon (*Oncorhynchus nerka*) in the Fraser River Estuary

David A. Levy and Allen D. Cadenhead

Abstract: Mobile acoustic surveys of 24-h duration were undertaken at Steveston Bend between 1991 and 1993, close to the time of maximum adult Early Stuart sockeye salmon (*Oncorhynchus nerka*) abundance in the Fraser River estuary. During each survey, extreme variations in the abundance and directional orientation of fish targets appeared to be related to local flow direction and velocities. Acoustic Doppler Current Profiler (ADCP) measurements of water currents adjacent to the fish monitoring site showed that the water column stratified into two vertical layers under certain tidal conditions, with an upper outflowing layer over a lower inflowing layer. Under strong flood or ebb conditions, most of the water column was, respectively, inflowing or outflowing. Fish abundance in the water column appeared to be related primarily to tidal current variations. Maximum fish abundance and upstream orientation occurred during flood tidal periods with upstream current flows. We conclude that migrations of Early Stuart sockeye salmon are synchronized with the tidal cycle for energetically efficient net upstream displacement through the Fraser River estuary.

Résumé : Nous avons effectué entre 1991 et 1993, vers la période d'abondance maximale de la remonte hâtive de saumons rouges (*Oncorhynchus nerka*) adultes de la rivière Stuart dans l'estuaire du Fraser, des relevés acoustiques mobiles d'une durée de 24 h à la courbe de Steveston. Pendant chaque relevé, les variations extrêmes de l'abondance et de l'orientation des poissons suivis semblaient reliées à l'orientation locale et à la vitesse du courant. Des mesures des courants effectuées au profileur acoustique (ADCP) près du site de surveillance des poissons ont révélé que la colonne d'eau se stratifiait en deux couches verticales à certains moments de la marée, avec une couche supérieure s'écoulant vers le large au-dessus d'une couche inférieure entrant dans l'estuaire. Dans de fortes conditions de flux et de reflux, la plus grande partie de la colonne d'eau s'écoulait, respectivement, vers l'estuaire ou vers la mer. L'abondance des poissons dans la colonne d'eau semblait reliée avant tout aux variations des courants de marée. On observait un maximum d'abondance du poisson et d'orientation vers l'amont dans les périodes de flux, où le courant était orienté vers l'amont. Nous en concluons que les migrations de la remonte hâtive de saumons rouges de la Stuart sont synchronisées avec le cycle maréal, ce qui permet un déplacement net vers l'amont, efficient sur le plan énergétique, dans l'estuaire du Fraser.

[Traduit par la Rédaction]

Introduction

Migratory aquatic animals frequently encounter opposing current flows that need to be stemmed to complete their migrations. For diadromous fish species, the upstream freshwater migration by juveniles (e.g., eels) and adults (e.g., salmon) can involve significant energy expenditures. For such species, there will be strong selection pressure for the fish to undertake energetically efficient migration by means of swimming velocity and (or) other behavioral adjustments (e.g., Webb 1975; Trump and Leggett 1980; Weihs 1984).

Upon entry into a river estuary, diadromous migrants encounter oscillating current flows resulting from the interaction of riverine and tidal currents. Previous studies indicate that estuarine migrants can utilize selective tidal stream transport to achieve net upstream displacement (McCleave and Kleckner 1982; Leggett 1984; McCleave and Wippelhauser 1987). Such behavior involves vertical migration in phase with the tidal flow to effect horizontal movement up the estuary. Migrants enter the water column under flood conditions when currents are flowing upstream, and leave the water column to maintain position close to the bottom substrate when the currents are flowing downstream. Similar behavior is also adopted by certain migratory marine fish species (Greer Walker et al. 1978; Arnold and

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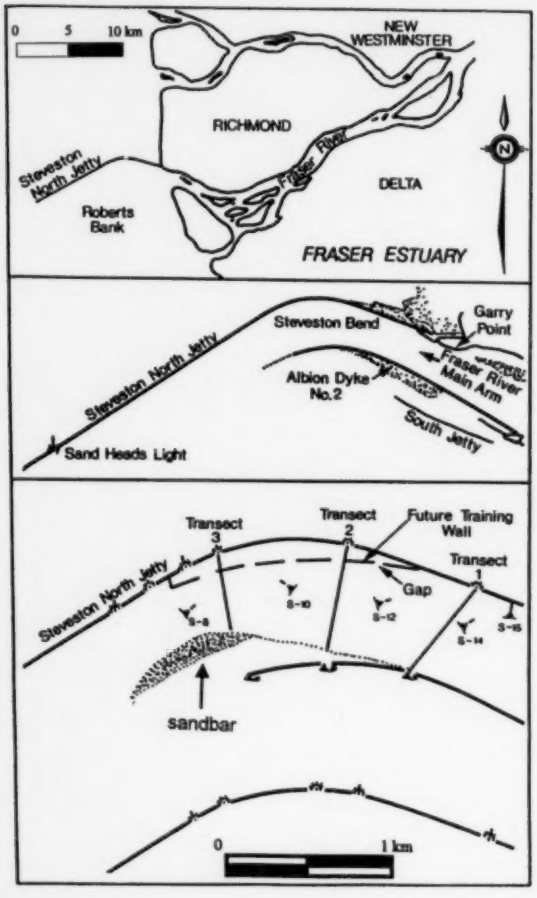
Fig. 1. Location of acoustic transects at Steveston Bend in the Main Arm of the Fraser River, relative to the location of the proposed training wall structure. Top: location of Steveston North Jetty in the Main Arm of the Fraser River estuary; middle: larger scale of Steveston North Jetty and Bend; bottom: still larger scale view of bend showing acoustic transect locations and channel buoys (S-8 to S-15).



Cook 1984), as well as other fish species to achieve estuarine penetration and retention of larvae and post larvae (Weinstein et al. 1980; Fortier and Leggett 1983).

Estuaries are important areas for human commerce, urban development, and navigation. For navigation purposes, estuarine channels are often dredged and maintained by means of training walls; navigation objectives frequently include maintenance of narrow, deep, fast-flowing and self-scouring estuarine channels. Modifications to estuarine flow patterns can have direct consequences on fish migrations, and there is a need for careful studies of fish movement patterns before impacts of such activities can be predicted (Quinn 1990).

Energetically efficient migration is critical for adult salmon that migrate long distances. In the Fraser River, British Columbia, sockeye salmon (*Oncorhynchus nerka*) from the Stuart River migrate more than 1000 km from tidewater to their spawning grounds. One of the Stuart River sockeye populations, the Early Stuart run, is the first major sockeye run to approach the Fraser River each year, and is managed independently from other sockeye populations due to its discrete migration timing (Woodey 1987). Early Stuart sockeye expend a large proportion of their energy reserves during migration (Idler and Clemens 1959), and in certain years, the migrants suffer high rates of prespawning mortality upstream of the Fraser Canyon (Gilhousen 1990).

Within the Fraser River estuary, a proposal to install a series of submerged groin structures at Steveston Bend (Fig.1) was predicted to increase ebb tidal flows by 0.3 m/sec under certain conditions (Western Canada Hydraulic Laboratories Ltd. 1988). Such flow increases could potentially impact the migration of Early Stuart sockeye salmon. The project is being monitored to detect changes in fish behavior associated with the installation of new structures (Levy 1991, 1992, 1993). The vulnerability of Early Stuart River sockeye and other Fraser River salmon runs to flow-induced migratory obstacles in the estuary depends critically upon their upstream migratory behavior. The objective of the present study was to determine whether Early Stuart sockeye salmon rely on selective tidal stream transport to accomplish their upstream migration through the Fraser River estuary, and secondarily, to investigate fish migratory behavior in relation to prevailing tidal currents.

Study area and methods

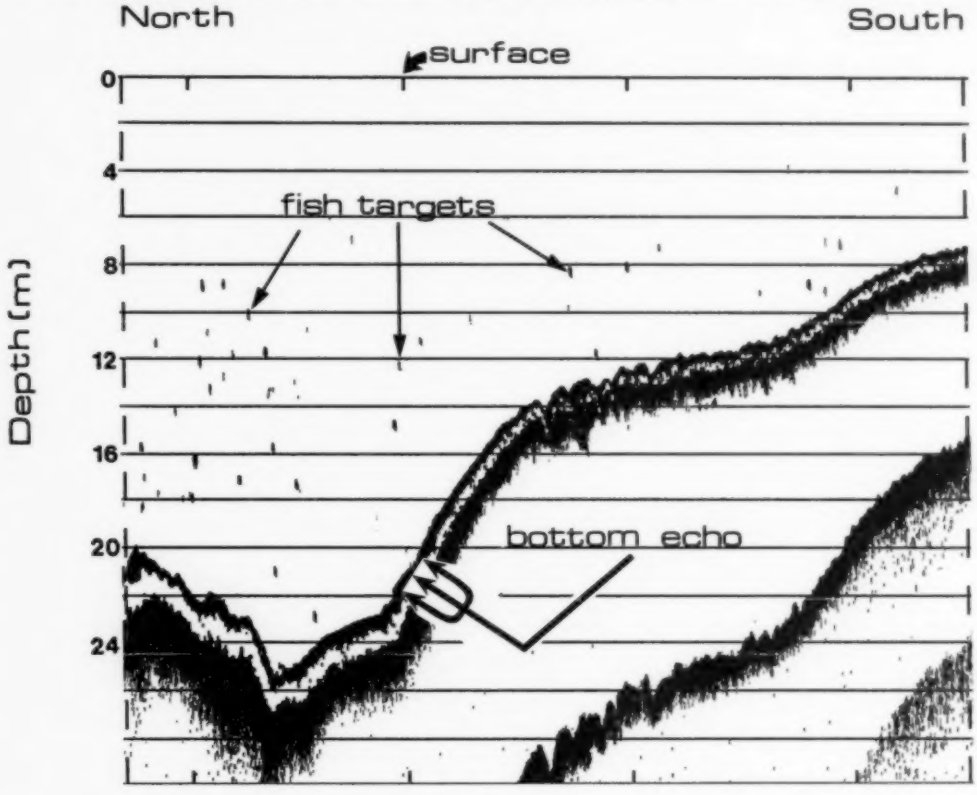
Study area

The study was conducted at the mouth of Main Arm of the Fraser River, a major Canadian salmon river (Northcote and Larkin 1989; Northcote and Burwash 1991), adjacent to the Steveston North Jetty at Steveston Bend (Fig. 1). The Main Arm of the river is the principal migration corridor for adult salmon returning to upstream spawning areas and is the traditional fishing location for a large gillnet fleet. The Main Arm is also the principal shipping channel for the Fraser River Port, which is maintained by training works and annual dredging programs. The river in the vicinity of Steveston Bend shows typical estuarine characteristics, with freshwater flow layered over a salt wedge (Hoos and Packman 1974). Flow discharge within the estuary is strongly influenced by mixed, semi-diurnal tidal fluctuations (Thompson 1981). The high volume of freshwater discharge from the Fraser River, coupled with tidal effects and estuarine mixing of fresh and seawater, results in complex flow conditions at Steveston Bend. A salt wedge penetrates into the Fraser River estuary upstream beyond the study site (Ages 1979, 1988; Ages and Woollard 1988).

Acoustic surveys

Our surveys were scheduled to correspond with the seasonal migration of Early Stuart sockeye salmon through the Fraser Estuary. Information on the timing of Early Stuart sockeye migrations was obtained from the Pacific Salmon Commission (600–1155 Robson St., Vancouver, BC V6E 1B5, Canada), which monitored Early Stuart

Fig. 2. Echogram taken on Transect 2 at 04:00 (PDST) on July 11, 1991. A total of 32 discrete echoes met criteria for fish target classification. Ticks on echograms applied by chart recorder at 1-min intervals.



sockeye catches in daily gillnet test fisheries in the Strait of Juan de Fuca and the Fraser River at Cottonwood Bar (adjacent to Annacis Island, 25 km upstream of the study site). Individual acoustic surveys were undertaken for durations of 24 h to evaluate diurnal variations in fish abundance. During 1991, two surveys were undertaken on July 10–11 and July 13–14, close to the peak Early Stuart sockeye abundance in this reach of the Fraser. During 1992 and 1993, the first survey occurred on June 15–16, prior to the arrival of Early Stuart sockeye, to estimate the frequency of "background" fish targets that were present prior to the arrival of Early Stuart fish. Two additional surveys were undertaken in mid-July to coincide with peak Early Stuart sockeye abundance in the study area.

In 1991, three acoustic transects, oriented perpendicularly to the flow of the Fraser River, were established in the vicinity of the proposed training wall (Fig. 1). These transects began at the Steveston North Jetty and followed a north to south line. The navigation channel is situated on the north side of the river, and is deeper than 24 m on transect 2 (Fig. 2). The south side slopes gradually towards the surface. Transects were located in the field using channel buoy markers situated on existing training walls. During 1992 and 1993, acoustic transects were undertaken only at transect 2; five transects were run each hour, back and forth across the river.

Acoustic sampling equipment and procedures

Mobile acoustic observations were taken from a 7 m survey vessel operating at 6–8 km/h. Transducers were suspended in a downlooking deployment, immediately beneath the water surface, by means of a pole mount stabilized by guy wires fore and aft. The acoustic equipment consisted of a 420-kHz, BioSonics model 105 dual beam echosounder, a 6°/15° dual beam transducer and cables, a Hitachi dual channel oscilloscope, and a Model 111 BioSonics thermal chart recorder. Power was supplied by a 1000-W gasoline generator in 1991 and by 12-V batteries in 1992–1993. The equipment was calibrated annually in the BioSonics calibration tank prior to the surveys. Measured performance values for the acoustic systems were as follows:

	1991	1992	1993
Source level (dB ‖ μPa at 1m)	212.26	219.36	219.23
Receiving sensitivity of wide beam channel (dB_v ‖ V/μPa at 1 m at 40 log *R* time varied gain)	−167.52	−176.99	−157.95

Echo sounder settings for the field surveys were 40 log R time varied gain, 0 dB receiving sensitivity, 0.4 ms pulse length, and a pulse rate of 20/s. Settings for the chart recorder included a range of 30 m and a chart speed of 1/16 mm per ping. To maximize the effective sampling volume of the system, the wide beam (15°) transducer was used to generate echo counts.

Since the acoustic systems utilized during the 3-year study differed slightly in transmission efficiency, receiving sensitivity, and transducer beam dimensions (measured during calibration), the chart recorder threshold was adjusted in 1992 and 1993 to equalize the sensitivity of the different systems. In 1991, the chart recorder voltage threshold was set at 800 mV. Using an average sockeye salmon fork length of 55 cm, the predicted dorsal-aspect target strength was −34 dB based on Love's (1977) regression equation that relates target strength to fish size:

$$(1)\quad TS_{\text{dorsal}} = 18.4 \log L - 1.6 \log f - 62.14$$

where TS_{dorsal} is the dorsal-aspect target strength (decibels), L is the fish length (centimetres), and f is the frequency of the acoustic system (kilohertz).

The effective beam angle of the wide-beam transducer employed in 1991 was estimated from the SONAR equation (Thorne 1983)

$$(2)\quad V_{\text{out}} = SL + TS + G_0 + 2B(\theta)$$

where V_{out} is the output voltage of the echo sounder, SL is the source level (i.e., intensity of a signal at a unit range from the transducer), TS is the target strength of the object, G_0 is the receiving sensitivity at gain 0, and $B(\theta)$ is the directivity pattern function. By setting V_{out} equal to the voltage threshold and solving for $B(\theta)$, the effective beam angle θ was extrapolated from the transducer directivity pattern. The calculated effective beam angle for a −34-dB object during the 1991 study was 13.5°. Using the SONAR equation above, a TS value of −34 dB, the measured SL values for 1992 and 1993 (above), a θ value of 13.5°, and extrapolating the value of $B(\theta)$ from the transducer directivity patterns, calculated V_{out} (voltage threshold) values were 1200 and 1350 mV during 1992 and 1993, respectively. Use of these calculated chart recorder thresholds effectively equalized the acoustic systems deployed between 1991 and 1993, so they were equally sensitive at detecting acoustic targets of a given size and target strength.

Fish targets were detected as discrete voltage pulses on the oscilloscope and formed well-defined traces on the chart recorder paper (Fig. 2). Fish targets were enumerated in 2-m depth strata. Fish distribution across the river was determined by enumerating the targets in four equal horizontal sections (quadrants) across the transect. To help distinguish fish from electrical noise, targets were enumerated only if they showed at least two consecutive voltage returns above the threshold (individual pulses were distinguishable on the echogram). Fish target densities ranged between 0 and 91 fish targets per transect.

In addition to the mobile observations taken on the three transects, a series of stationary observations were made at channel marker S-15 (Fig. 1) at hourly intervals during the surveys. The transducer was fixed at a 30° upstream angle so fish directional orientation (upstream, downstream, or nondirectional) could be determined from the angle of chart marks caused by fish movements (recorded as change-in-range measurements on echograms). Examples of angled echo traces produced by fish moving through the sonic field of an angled transducer are shown in Levy (1991).

Echo target frequencies provided indices of relative fish abundance at different sampling times and locations. For analysis of fish vertical distribution within the water column, target frequencies were adjusted to account for the different sampling volumes in adjacent depth strata (due to the conical shape of the acoustic beam). In each 2-m depth stratum, fish target frequencies were scaled as follows:

$$(3)\quad \text{Relative fish number} = \frac{\text{number of fish targets}}{2R \tan(\theta/2)}$$

where R is the range (metres), and θ is the effective beam angle of the transducer.

Species composition

During 1991 and 1992, fish species composition was determined from commercial and test-fishing gillnet catches near the study site (Levy 1991, 1992). Species composition in 1993 was determined using a gillnetting program that operated adjacent to transect 2. Commercial-type gillnets were fished on three dates during flood tides close to the time of the acoustic transects. On each date, gillnets of mesh sizes 19.1 and 12.7 cm (corresponding to "chinook" and "sockeye" mesh sizes) were fished end to end in series. On the first two dates standard commercial nets (60 mesh deep) were fished throughout the upper 10 m (approx.) of the water column, while on the final date deeper gillnets (90 mesh deep) covering the upper 15 m (approx.) of the water column were deployed.

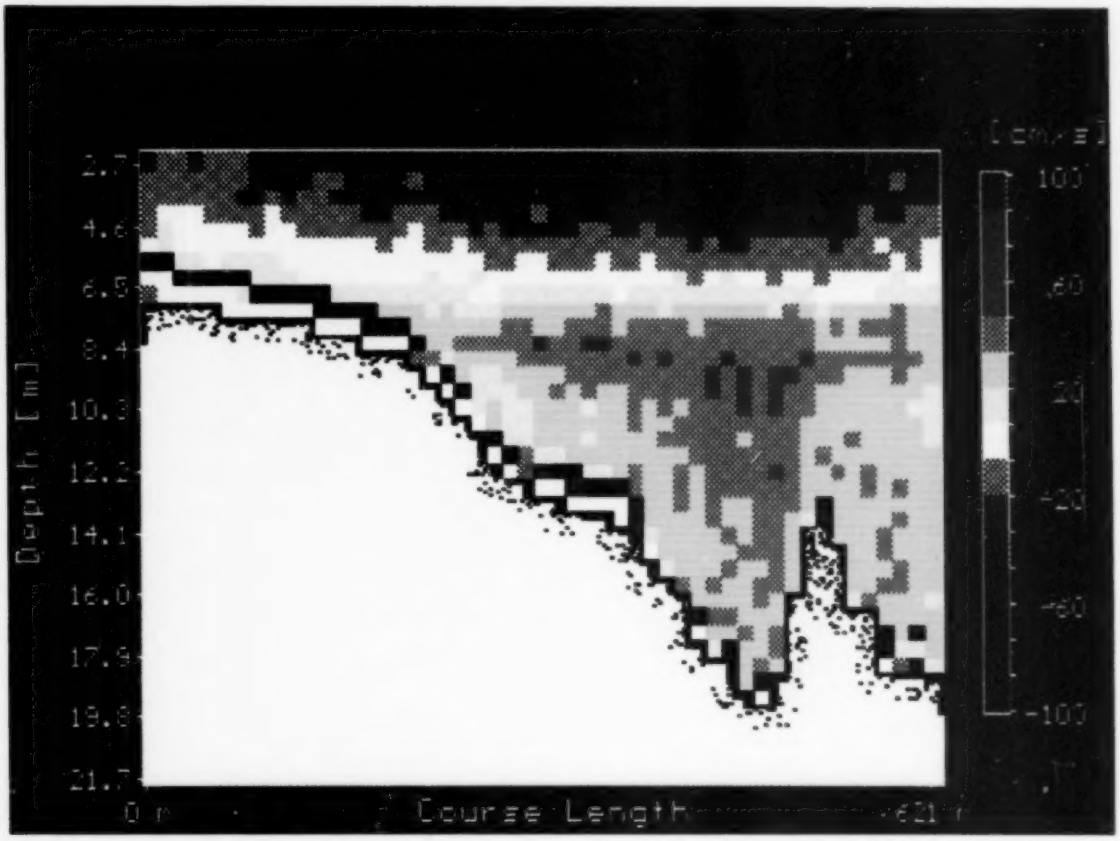
Current velocity

To measure the current velocity structure of the water column during 1992 and 1993, an Acoustic Doppler Current Profiler (ADCP) device was deployed from a separate vessel on the fish survey dates, over an 11-transect grid spanning the study area. ADCP results for the transect adjacent to fish survey transect 2 were analysed for the present study, to measure current velocity profiles and their temporal variability, at the water depths where fish concentrated.

Briefly, the ADCP device measures a Doppler shift in reflected high frequency sound pulses that are transmitted from four aligned transducers. Sound-scattering particles (including organisms) in the water column provide the echoes that are received by the transducers, converted to analogue signals, and processed and stored in digital format (in *.pcx data files) on a ship-board microcomputer. The measured Doppler shift can be stored and displayed as a colour image that depicts water current velocity across different depth strata and horizontal locations. Additional information about the ADCP device is contained in RD Instruments (1989). In the office, the stored data files were transferred to CorelDraw 4.0 and printed on a Hewlett-Packard PaintJet XL300 colour printer.

Colour echograms from the ADCP device were displayed as figures (e.g., Fig. 3) in which the horizontal axis shows the position across the transect from the south (left side) to north (right side). The vertical axis shows the

Fig. 3. Representative echogram from the Acoustic Doppler Current Profiler (ADCP) device showing a cross section of the Fraser River water column in the vicinity of Steveston Bend at 00:43 on July 15, 1993. Colours depict current velocity according to the scale on the right.



depth from the face of the transducer (i.e., 0.5 m below surface) to the river bottom. Current velocities are represented by the colours on the scale shown adjacent to the echogram. Positive water velocities (yellow, red) represent flow in an easterly direction (i.e., upstream), while negative water velocities (blue, purple) represent flow in a westerly direction (i.e., seaward). Black areas on the echogram indicate the presence of river bottom or bad (high voltage) echo returns. Figure 3 depicts a bidirectional flow pattern, with surface (upper 5 m) waters outflowing over underlying waters (6–20 m) that are flowing upstream.

To correlate current velocities with local tidal fluctuations at Steveston Bend, water levels were measured on a tidal gauge adjacent to the fish monitoring transect at 30-min intervals. Tidal elevations are relative to the 0-m level on Canadian Hydrographic Survey charts (0 m represents mean lowest low water). These water levels provided a benchmark for comparing water velocity and flow conditions.

Results

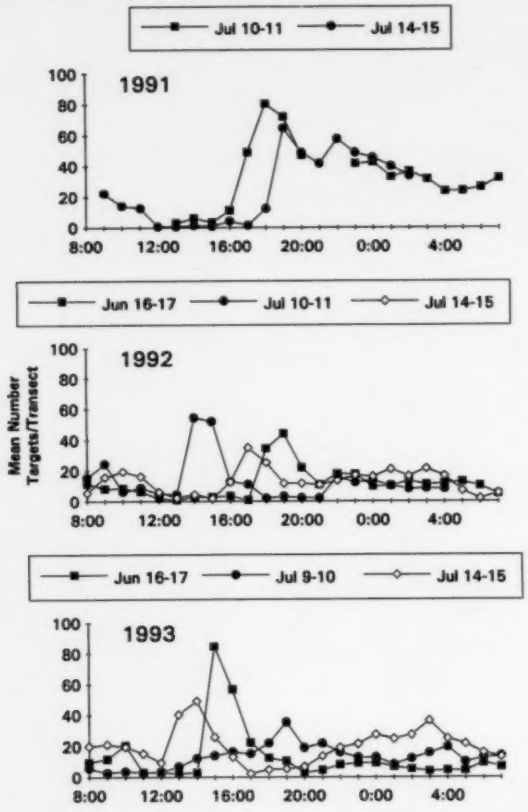
Fish species composition at Steveston Bend during the 1993 surveys was inferred from the gillnet catch results:

	June 21, 1993	July 8, 1993	July 15, 1993
No. of min fished	164	120	65
Sockeye	–	34	17
Chinook	8	6	1
Other	–	–	1 dogfish, 1 staghorn sculpin

These fish catches indicate that Early Stuart sockeye salmon were numerically predominant at Steveston Bend during the July surveys. Chinook salmon (*Oncorhynchus tshawytscha*) were also present during June and July surveys. Other data, which confirm that adult sockeye salmon are the predominant fish species present in this region of the Fraser Estuary in early to mid July, include gillnet catches from an adjacent gillnet fishery, as well as results from gillnet test fishing undertaken by the Pacific Salmon Commission (Levy 1992).

Comparisons of echo target frequencies indicated that peaks in fish abundance occurred at different times over

Fig. 4. Average fish target frequency per transect, for locations adjacent to Steveston Bend in the Fraser River during 1991, 1992, and 1993. All times shown as Pacific Daylight Savings Time (PDST).



the 24-h cycle (Fig. 4). Highest numbers were recorded in the early evening in 1991, and in the mid-afternoon to early evening period in 1992 and 1993. There were also periods during every survey when fish target abundance was low (less than 10 targets per transect).

It was initially anticipated that acoustic surveys during mid-June of 1992 and 1993 would result in lower echo target frequencies than surveys conducted in early to mid July, close to the peak migration of Early Stuart sockeye salmon in the Fraser River estuary. Such was not the case (Fig. 4): mid-June surveys resulted in similar (1992) or higher (1993) echo target frequencies than mid-July surveys. Based on gillnetting results (above), chinook salmon comprised a large fraction of the mid-June echo targets during 1993.

The hourly orientation of fish (upstream and downstream) adjacent to the study site (Fig. 5) was determined during the surveys by examining the shape of echo marks produced when fish swam through the sonic field of an angled, stationary transducer. During most observations, fish targets were strongly (usually 100%) oriented either upstream or downstream, with major shifts (reversals) evident in directional preference (Fig. 5). For example, on July 14–15, observations between 13:00 and 00:00 indicated the majority of fish targets travelling in an upstream direction. Between early morning through to mid-day periods on July 15 the majority of fish targets were oriented in a downstream direction. Several of the July late-afternoon and evening observations showed targets oriented both upstream and downstream, indicative of transition periods when orientation direction was changing. Similar temporal shifts in the directional preference of fish were observed in 1991 (Levy 1991) and 1992 (Levy 1992).

Depth distribution profiles for the 1993 transects (Fig. 6A) indicated sharp peaks in fish abundance at 7- to 9-m depths. Mean fish depths ranged between 5 and 14 m (Fig. 6B), with overall mean depths of 9.4, 9.4, and 9.0 m on June 16–17, July 9–10, and July 14–15, respectively. Similar patterns of vertical fish distribution were observed at the study site in both 1991 and 1992.

Comparison of the echo target frequency in four horizontal quadrants across the transect (Fig. 7) suggested a skewed fish distribution, with most fish targets occurring in quadrants 1 and 2, closest to the training wall. This northern section of the river is deeper than the southern portion (quadrants 3 and 4; see bottom profile in Fig. 2). Similar patterns of horizontal distribution were observed in both 1991 and 1992.

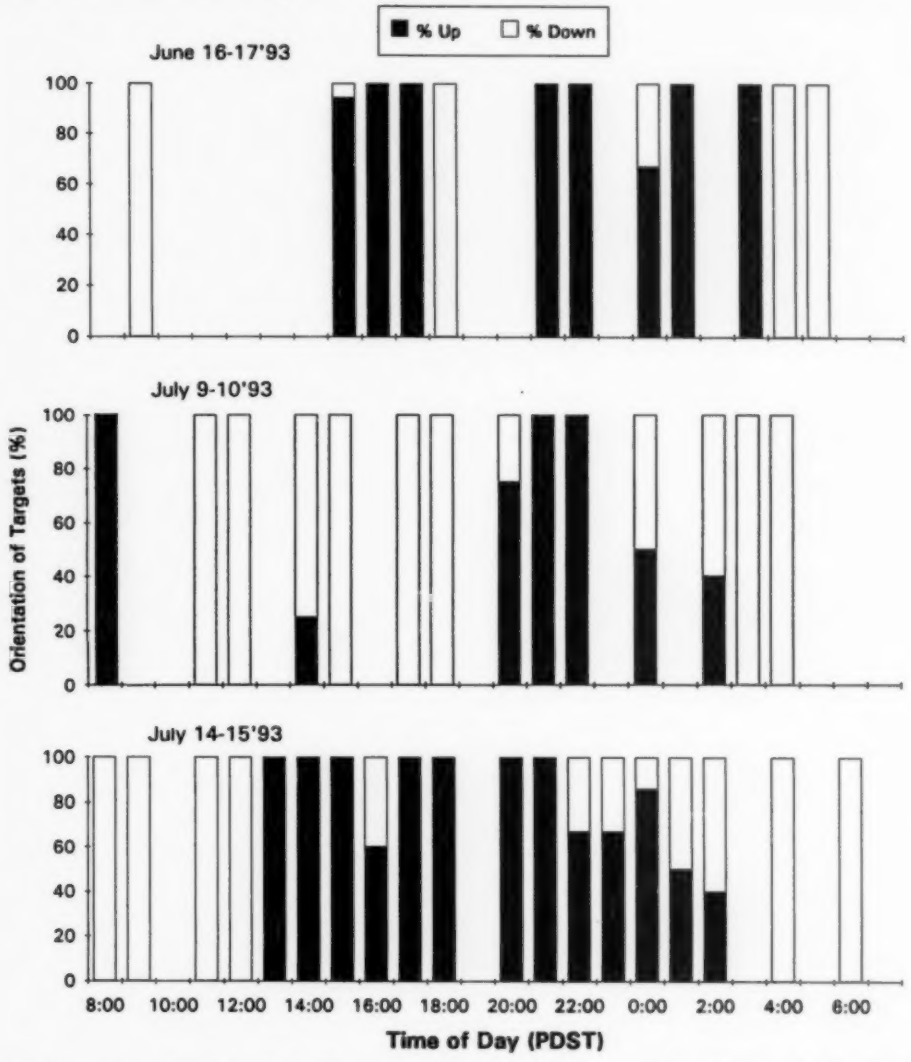
On July 9–10, the first ADCP transect (10:53) indicated outflowing currents layered over a lower inflowing countercurrent (Fig. 8). Between 12:39 and 15:46, the entire water column was outflowing (blue/purple), with the exception of a thin countercurrent layer immediately above the bottom (yellow). Between 16:43 and 17:37, the lower upstream-flowing layer increased in spatial magnitude and by 18:35, the entire water column was flowing upstream (yellow/red). Upstream flows were maintained until 00:45, when a surface outflowing current developed (blue). The outflowing surface layer increased in depth until 04:01, when most of the water column was outflowing. At 05:45 and subsequently, the lower upstream flowing layer increased in thickness, and by 08:32, most of the water column was flowing upstream. At this time, surface waters closest to the training wall were flowing upstream (yellow), while those in the central portion of the river were outflowing (blue). Similar patterns of flow were also documented on June 16–17, 1993 and July 14–15, 1993 (Levy 1993), and July 10–11, 1992 and July 14–15, 1992 (Levy 1992), although the timing of flow events varied.

A comparison of fish behavior and current flow conditions adjacent to Steveston Bend (Fig. 9) suggests that peak fish numbers and upstream orientation coincided with upstream flows, when tidal currents are favourable for upstream migration. Periods of flooding tides and upstream flows (defined as periods of the day when over 50% of the water column contains upstream flowing currents) coincided with peak target abundance and upstream orientation. The temporal pattern of fish migration also shifted on the different observation dates in phase with the shift in timing of the semi-diurnal tidal cycle (Fig. 9).

Discussion

The present study suggests that Early Stuart sockeye salmon utilize selective tidal stream transport to undertake their

Fig. 5. Percentage of upstream- and downstream-oriented fish targets adjacent to channel buoy S-15 in the Fraser River during 1993.



upstream migration through the Fraser River estuary. The behavior involves the exploitation of flood tidal currents that are directed upstream, and the avoidance of downstream current flows associated with ebb tidal stages. By concentrating at mid-water depths during flood tide within the deeper sections of the river channel, the fish maximize their upstream displacement through the estuary, consistent with upstream migration objectives.

Inflowing tidal currents may have provided a trigger for upstream migration by Early Stuart sockeye salmon. During unfavourable flow conditions induced by ebbing tides, relatively few echo targets were present in the water column. Under such flow conditions, we suspect that the fish were holding in position close to the river bottom, where they were undetectable by a surface-oriented, down-looking transducer.

Early Stuart sockeye cover a long migration distance in freshwater (>1000 km) between the Fraser estuary and their spawning grounds in the Stuart River. They do not feed in freshwater, nor do they have a large amount of surplus stored energy that can be allocated to upstream migration (Idler and Clemens 1959). Under such conditions, there will be strong selection pressure on Early Stuart sockeye to both swim efficiently and adopt migration behavior that minimizes energy expenditure. Theoretical calculations by Weihs (1978) suggest that significant energy savings, on the order of 40–90%, can be obtained by migrating fish that adopt selective tidal stream transport. Three different categories of fish swimming behavior are commonly recognized (Webb 1975; Beamish 1978). These include sustained swimming (velocities that can be maintained for periods longer than 200 min without muscular

Fig. 6. (A) Fish vertical depth distribution profiles shown as the proportion of the total estimated population and (B) mean depth of fish targets as a function of time of day on three sampling dates during June and July, 1993.

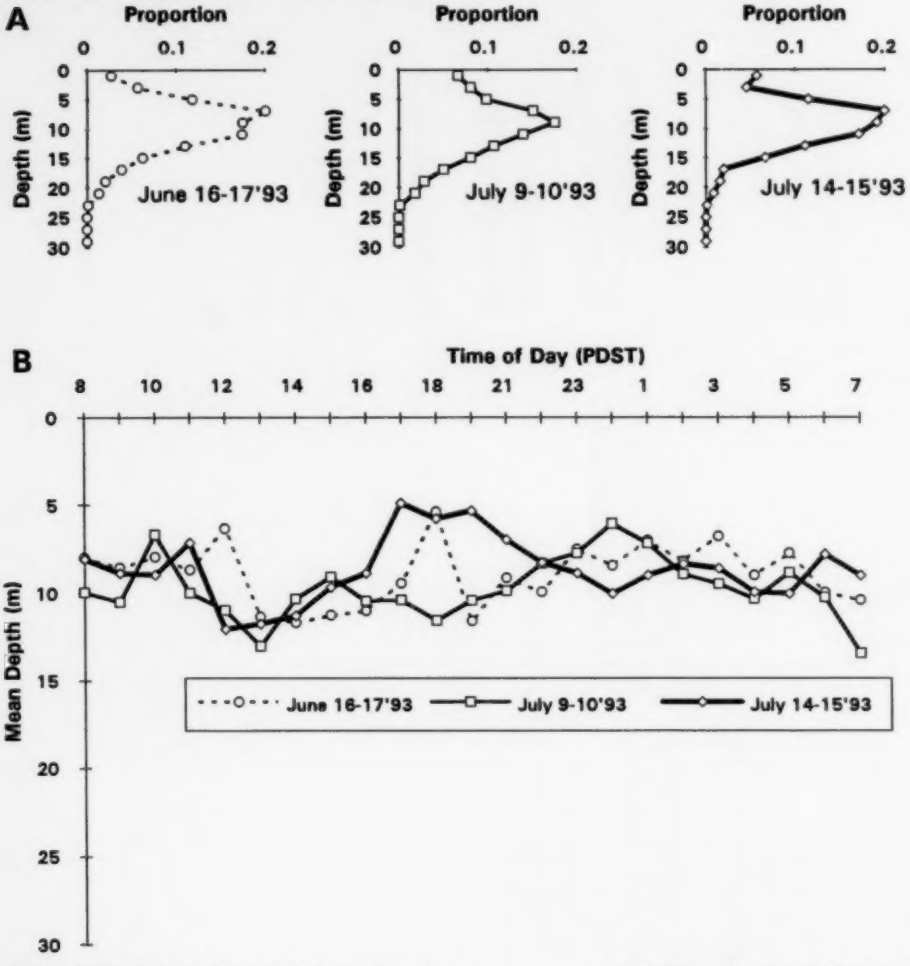
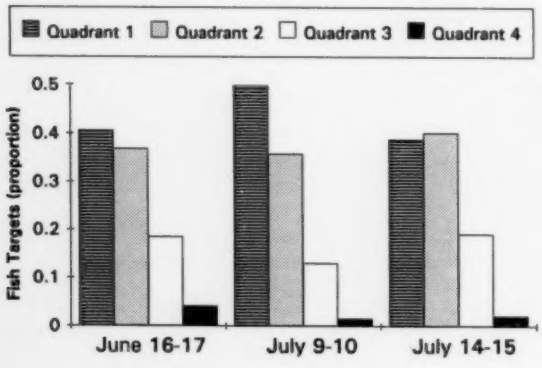


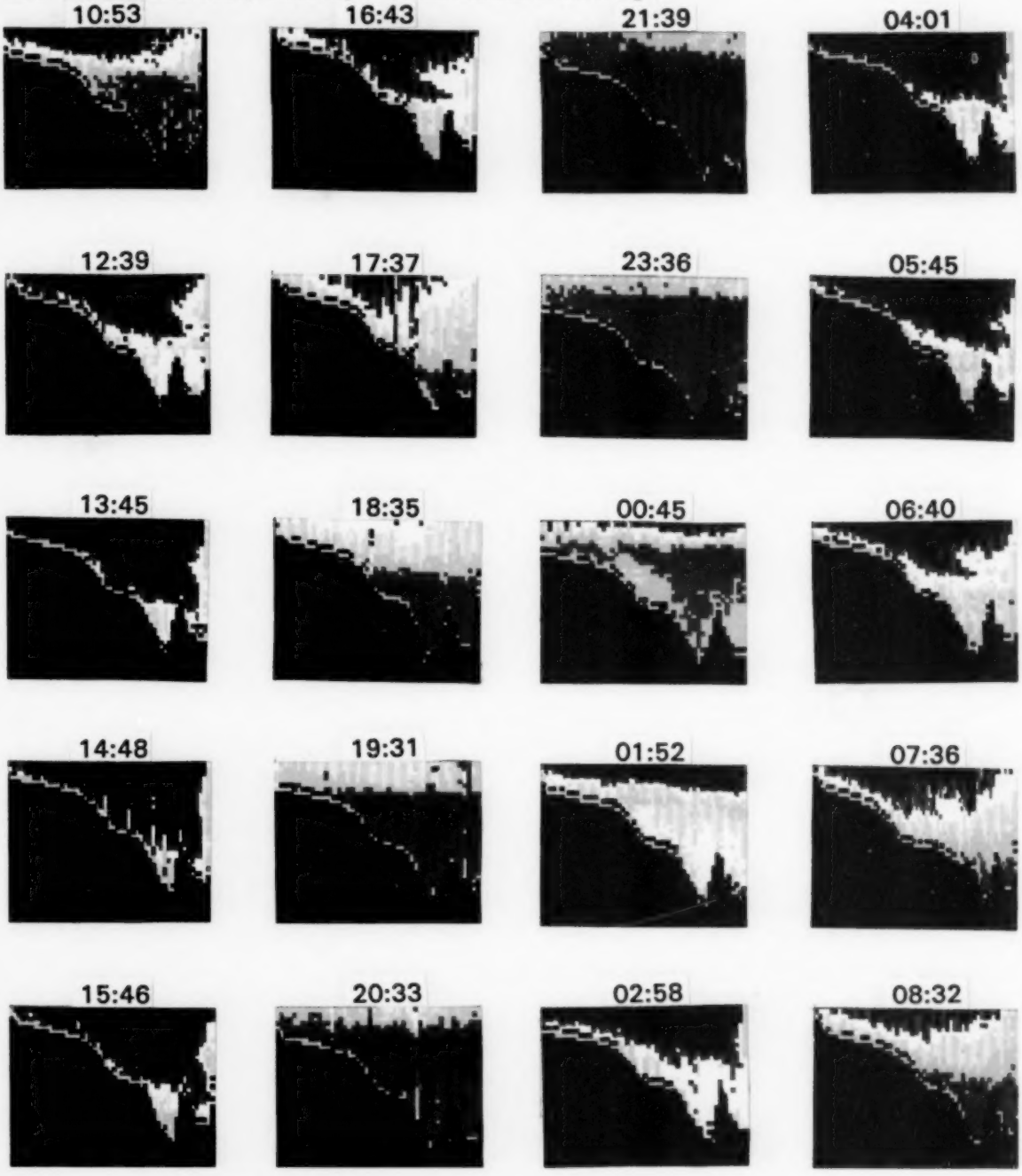
Fig. 7. Relative proportions of fish targets in four equidistant sections of transect No. 2 running from north (quadrant 1, adjacent to Steveston North Jetty) to south (quadrant 4, adjacent to Albion Dyke no. 2) during 1993.



fatigue), prolonged swimming (velocities that can be maintained between 15 s and 200 min and result in fatigue), and burst swimming (high activity levels maintained for less than 15 s). The maximum sustained swimming velocity for sockeye salmon is about 1.3 m/s (Brett 1982). The energetically optimal swimming speed for an upstream migrant that encounters detectable, oscillating currents (Trump and Leggett 1980), such as those that occur in the Fraser River estuary, is about 1 body length per second, well below the maximum sustained swimming velocity of sockeye. By undertaking selective tidal stream transport and migrating at sustained swimming velocities, Early Stuart sockeye could effectively minimize their energy expenditure per unit distance as they migrate through the Fraser River estuary.

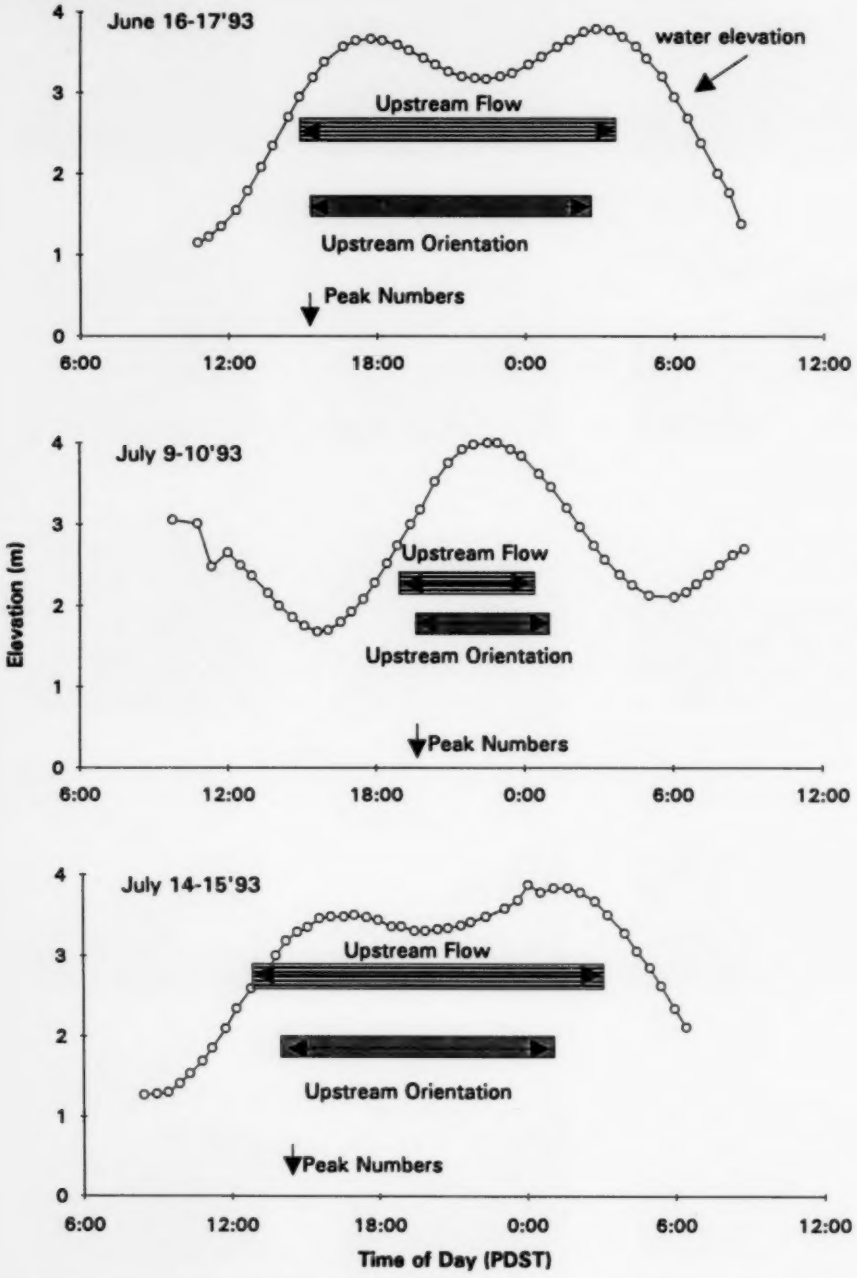
It is not presently clear at what point during their upstream migration that Early Stuart sockeye undertake selective tidal stream transport. Ultrasonic tracking studies of adult sockeye in B.C. coastal waters have shown that

Fig. 8. Acoustic Doppler current profiles adjacent to Steveston Bend on July 9–10, 1993 (numbers above echograms depict the time of the transect, PDST). Colour key for current velocities shown on Fig. 3.



the direction, swimming speed, and depth of migration are discontinuous (Madison et al. 1972; Stasko et al. 1976; Quinn and terHart 1987). Certain runs of Fraser River sockeye delay their upstream migration in the Strait of Georgia for about a month (e.g., Adams River sockeye, Gilhousen 1960). In the Skeena River estuary, adult sockeye fitted with ultrasonic transmitters apparently drifted in tidal currents and made little upstream headway over the periods they were tracked (Groot et al. 1975). Fraser River sockeye orient towards the thermocline and halocline while

Fig. 9. Composite graphs showing timing of diel abundance peak (Fig. 4), upstream orientation of acoustic targets (Fig. 5), and upstream flow conditions (Fig. 8 and Figs. 11–12 in Levy 1993) in relation to measured water level elevation above mean lowest low water at Steveston Bend (open circles).



in B.C. coastal waters (Quinn and terHart 1987; Quinn et al. 1989), but the tracks of tagged sockeye do not suggest selective tidal stream transport by sockeye in the Strait of Georgia. Sockeye salmon in the Fraser River were previously enumerated as they migrated past acoustic devices at Mission, B.C., 90 km upstream of the Fraser estuary (Levy et al. 1991). There was extreme hour-to-hour variation in the estimated number of sockeye enumerated, which may reflect a tidal influence, since the river is partially tidal at this site. Further upstream from Mission, tidal

effects are negligible and other factors undoubtedly serve as migration cues.

The question of which sensory mechanism may be involved in selective tidal stream transport has been addressed by McCleave and Kleckner (1982) who evaluated possible olfactory, rheotactic, and electrical cues, as well as circatidal clocks to provide a mechanism for selective tidal stream transport in glass eels (juveniles) of the American eel (*Anguilla rostrata*). No definitive conclusions were reached, and the possibility was also raised that multiple sensory cues could also be involved, e.g., odour to provide the cue to "lock on" to the proper tide, and flow reversal (a rheotactic cue) to time an ascent into the water column. Salmon are highly sensitive to the set of sensory cues listed above (Smith 1985); further upstream beyond tidewater, upriver migration probably involves both olfactory stimuli and rheotactic responses (Johnsen and Hasler 1980).

The ADCP technology utilized during the present study can be potentially applied to a wide set of practical and theoretical problems in aquatic ecology, ranging from precise definition of effluent plumes and current velocity changes in relation to riverine and coastal structures, through to the testing of predictions from fish migration models. Recently reported applications include studies to monitor tidal currents (Vennell 1994) and sediment plumes (Reichel and Nachtnebel 1994). Future applications in the field of fisheries biology could be undertaken by means of both stationary, in situ deployments, as well as by means of mobile surveys in aquatic areas of managerial or scientific interest.

Selective tidal stream transport occurs in a variety of marine (Greer Walker et al. 1978; Arnold and Cook 1984) and diadromous (McCleave and Kleckner 1982; this study) fish species. Atlantic salmon (*Salmo salar*) can reside temporarily in an estuary for several months prior to upstream migration (Brawn 1982). During active estuarine migration, Atlantic salmon swimming behavior is consistent with selective tidal stream transport (Stasko 1975), and evidently involves drifting in flood tidal currents, and stemming of ebb tidal currents. Although the behavior has not been widely documented in Pacific salmon, we believe that selective tidal stream transport is probably undertaken by other species as well as sockeye and probably occurs in numerous river estuaries covering the geographical range of *Oncorhynchus* spp.

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Comparison of intermittent and continuous exposure to mercuric chloride in rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), and the fathead minnow (*Pimephales promelas*)

R.D. Handy

Abstract: Rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), and the fathead minnow (*Pimephales promelas*) were exposed continuously or intermittently (24-h exposure : 24-h recovery) to a nominal peak concentration of 3 $\mu g \cdot L^{-1}$ mercuric chloride for 120 h. There were no differences in the target organs or the distribution of the toxicant within internal organs between the two exposure regimes. Mercury concentrations in the tissues of intermittently exposed fish were less than those of continuously exposed fish. The lower mercury concentrations in the intermittently exposed groups arose from reduced or negligible accumulation during recovery periods rather than mercury excretion. The accumulation of mercury during intermittent exposure is roughly proportional to the exposure duration, and could therefore be predicted from a continuous exposure of equivalent total exposure duration. This proportionality exists when (1) peak concentrations of mercury are the same in both regimes, and (2) the recovery periods are short compared with the biological half-life for mercury.

Résumé : La truite arc-en-ciel (*Oncorhynchus mykiss*), le cyprin doré (*Carassius auratus*) et la tête-de-boule (*Pimephales promelas*) ont été exposés de façon continue ou intermittente (24 h d'exposition : 24 h de récupération) à une concentration nominale maximale de 3 $\mu g \cdot L^{-1}$ de chlorure de mercure pendant 120 h. Il n'y avait pas de différences dans les organes ciblés ni dans la distribution du toxique parmi les organes internes entre les deux régimes d'exposition. Les concentrations de mercure dans les tissus des poissons exposés de façon intermittente étaient inférieures à celles des poissons exposés de façon continue. Ces concentrations plus faibles s'expliquent par l'accumulation réduite ou négligeable de mercure pendant les périodes de récupération plutôt que par l'excrétion de ce métal. L'accumulation du mercure pendant l'exposition intermittente est grossièrement proportionnelle à la durée de l'exposition, et pourrait donc être prédite à partir d'une exposition continue dont la durée totale serait équivalente. Cette proportionnalité existe quand (1) les concentrations maximales de mercure sont les mêmes dans les deux régimes, et (2) les périodes de récupération sont courtes par rapport à la période biologique du mercure.

[Traduit par la Rédaction]

Introduction

In the last decade intermittent pollution has been identified as an important mode of contamination in the aquatic environment. Research in this new area of ecotoxicology has focused principally on agrochemicals, and consequently only a few metals have been investigated (Cu, Seim et al. 1984; Cd, Pascoe and Shazili 1986; Al, Siddens et al. 1986; Cd, Cu, Handy 1992; reviews McCahon and Pascoe 1990; Handy 1994). Mercury is a particularly important intermittent pollutant because it contaminates aquatic ecosystems as trade effluent or waste waters, which are often highly variable in volume and mercury content (Nakanishi et al. 1989; Pfeiffer et al. 1989; Gonzalez 1991). The principal water soluble compound in anthropogenic wastes is mercuric chloride, which is also toxic to fish (Beneš and Havlík 1979). Despite these observations, mercury accumulation by fish during intermittent exposure regimes has not been investigated.

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There is a large body of literature on continuous exposure to mercury compounds (Macleod and Pessah 1973; McKim et al. 1976; Snarki and Olson 1982; Huckabee et al. 1979; Spry and Wiener 1991). These studies show that waterborne inorganic mercury compounds enter the blood of unfed fish via the gills (Olson et al. 1973). There is some debate on how inorganic mercury crosses the gills (Olson et al. 1973; Huckabee et al. 1979; Paulose 1989; Stinson and Mallatt 1989), and what proportion of the toxicant is trapped in the glycocalyx–mucus layer on the branchial surfaces (McKone et al. 1971; Lock and van Overbeeke 1981; Pärt and Lock 1983; Paulose 1989). Inorganic mercury may have be methylated to the rapidly absorbed methylmercury form on mucus surfaces on fish (Lock and van Overbeeke 1981; Rudd et al. 1983; Winfrey and Rudd 1990). Once mercuric chloride has entered the blood it accumulates in the liver and kidney, but is mainly stored in the muscle as methylmercury (Olson et al. 1973; Weisbart 1973; Misra et al. 1989; Harrison et al. 1990; Bloom 1992).

It is presently unknown whether the dynamics of mercury accumulation are altered by rapid multiple exposures. Consequently bioaccumulation and toxicity cannot be predicted for these environmentally realistic events. The objective of this study was to provide the first detailed account of mercury accumulation in fresh water fish exposed intermittently to the metal. The lack of experimental data also prompted the use of several species of freshwater fish (rainbow trout, goldfish, and fathead minnows) to see if a general pattern of accumulation could be established for intermittent exposure. These experiments were not, however, intended as an interspecies comparison of mercury accumulation.

Methods

Experimental animals

Rainbow trout (*Oncorhynchus mykiss*), goldfish (*Carassius auratus*), and the fathead minnow (*Pimephales promelas*) weighing 137.1 ± 7.3, 62.8 ± 2.4, and 0.47 ± 0.02 g, respectively (mean ± SE, $n = 28$) were obtained from commercial suppliers. Fish were maintained in holding aquaria for at least 2 weeks before use and were supplied with flowing, aerated Dundee tap water containing Na^+, 4.11 ± 0.05; K^+, 0.76 ± 0.01; Ca^{2+}, 6.29 ± 0.09; Mg^{2+}, 1.45 ± 0.01; Hg < 0.000001; Cl^-, 4.58 ± 0.04 $mg \cdot L^{-1}$ (mean ± SE, $n = 10$) at pH 7.4–7.8 and 15 ± 1°C. All animals were fed daily; trout received commercial pellets containing 0.170 ± 0.019 mg of mercury/kg dry weight of food (mean ± SE, $n = 8$). Goldfish and minnows were fed on tropical fish flakes (mercury content not measured). Fish stocks were not fed for 2 days before transfer to experimental tanks or during the subsequent experiments.

Experimental protocol

Eighty-four rainbow trout, goldfish, or fathead minnows were divided into two 400-L experimental tanks. Each tank was supplied with aerated Dundee tap water, flowing at a rate of 1.06 $L \cdot min^{-1}$ from a constant head device. Fish spent 24 h in the experimental system before dosing with a stock solution of 1 $g \cdot L^{-1}$ mercury as $HgCl_2$ (acidified with "Spectrosol" sulphuric acid) delivered via a peristaltic pump (5 $mL \cdot min^{-1}$). This gave a nominal mercury concentration of 3 $\mu g \cdot L^{-1}$ at pH 7.0 during continuous exposure.

One tank of fish was continuously exposed to mercury for 120 h, while the other was subjected to three intermittent pulses of mercury, for each of the species studied. Each intermittent exposure lasted 24 h and was separated by 24-h periods of clean water. A typical profile is illustrated in Fig. 1. Water total mercury concentrations were typically below 1 $ng \cdot L^{-1}$ at the start of each experiment, but reached a 24-h average of 3.04 ± 0.12 $\mu g \cdot L^{-1}$ (mean ± SE, $n = 17$) during continuous exposure. Intermittent exposure typically produced a 24-h average total mercury concentration of 2.817 ± 0.27 $\mu g \cdot L^{-1}$ ($n = 13$) during exposure phases compared with 0.65 ± 0.125 $\mu g \cdot L^{-1}$ ($n = 12$) in recovery periods (Fig. 1). This produced an average intermittent exposure mercury concentration of 1.95 ± 0.87 $\mu g \cdot L^{-1}$ ($n = 5$) for the 120-h duration of the experiment. Water pH remained neutral; typical values were 6.93 ± 0.07 (mean ± SE, $n = 11$) and 6.91 ± 0.07 ($n = 13$) for continuous and intermittent exposure, respectively. Water temperature was 15 ± 1°C throughout the experiments.

Out-flowing water samples (200 mL) were collected hourly during the first 12 h of each experiment, then every 12 h for the remainder of the exposure period. Water samples were acidified with a few drops of 0.1 N (0.2 M) Spectrosol sulphuric acid and stored at 4°C for up to 1 week before mercury determination (see below). Water pH and temperature were monitored using a low conductivity pH electrode and thermocouple connected to a pH microprocessor (Russell pH, Auchtermuchty, Scotland).

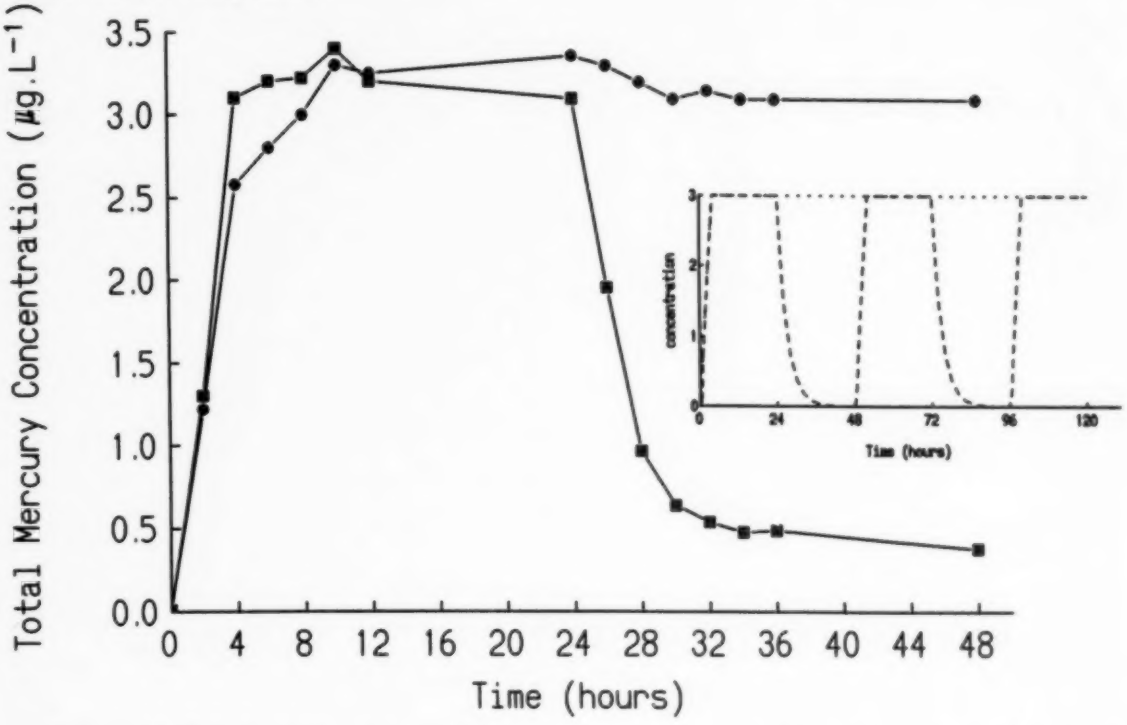
Dissection procedures

Groups of six goldfish or seven trout were removed from each exposure regime for dissection and mercury analysis at 24-h intervals; pre-exposure controls were collected at 0 h. Fish were netted from experimental aquaria and anaesthetized with a lethal dose of benzocaine (0.32 $mg \cdot L^{-1}$). The exterior of the fish was blotted dry and whole blood samples were immediately collected into heparinized syringes via cardiac puncture. Whole blood samples were then transferred to preweighed tubes. Then samples (0.4–1 g) of muscle, whole gill, liver, and kidney were sequentially removed from each fish and collected separately into preweighed plastic tubes. The fish carcass after tissue sampling was homogenized in an electric mincer and a subsample (approximately 4 g) of macerated fish was placed in a preweighed 30-mL tube. Separate dissection instruments were used for each organ and all instruments were acid washed and deionized between each group of fish to avoid cross contamination. The electric mincer was similarly cleaned between each group of fish. The fathead minnows were not dissected for internal organs because of their small size; instead fish were anaesthetized as described, blotted dry, and placed in 13-mL plastic tubes.

Sample analysis

Tissue and water samples were analysed for mercury by cold vapour atomic absorption spectrophotometry (CV-AAS), using a Pye Unicam SP9 and Philips cold vapour kit. All reagents were Spectrosol grade and glassware

Fig. 1. Total mercury concentrations in the exposure medium during one cycle of intermittent (■) and continuous (●) exposure to mercuric chloride. Values are means of triplicate water samples, standard errors are too small to be visible. Inserted graph indicates the number of repeated exposures in 120 h, dashed and dotted lines represent intermittent and continuous exposure, respectively.



was immersed in $K_2Cr_2O_8$–H_2SO_4 solution overnight, then acid washed and deionized before use. The method of analysis was supplied by Philips Analytical (Cambridge) and is similar to cold vapour methods reported elsewhere (Lajunen et al. 1985).

Samples of whole blood, muscle, gill, liver, and kidney (about 0.5–1 g) from trout or goldfish, as well as whole fathead minnows were digested in 5-mL aliquots of concentrated (18 N (36 M)) sulphuric acid in sealed tubes at 70°C for 2 h. Digests were then cooled and transferred to 100-mL Pyrex flasks, before carefully adding 50 mL of 6% potassium permanganate. Flasks were covered with watch glasses and heated for a further 2 h at 70°C and cooled; the excess permanganate was removed with 15 mL of 20% hydroxylammonium chloride. Mercury was liberated from solution with 2 mL of 10% $SnCl_2$. Subsamples of macerated fish remains were similarly treated, except that tissues were digested in 10 mL of concentrated sulphuric acid.

The total mercury content of 50-mL water samples was also derived following the same methods for fish tissues, except that the potassium permanganate was added as powder rather than solution (3 g per flask).

The total mercury contents of samples were determined against acidified, matrix matched, mercuric chloride standards prepared using deionized water. Analytical precision was ±5%. The mercury detection limit was 0.1 ng per flask, equivalent to 0.1 $ng \cdot g^{-1}$ for blood, muscle, gill, liver, and kidney samples or 0.5 $ng \cdot g^{-1}$ for fish carcass.

Mortalities

Fish were observed daily for signs of toxicity (loss of equilibrium, motility). Fish were considered dead when opercular movements ceased. Dead animals were removed from the tanks and analysed for mercury content as described for live fish.

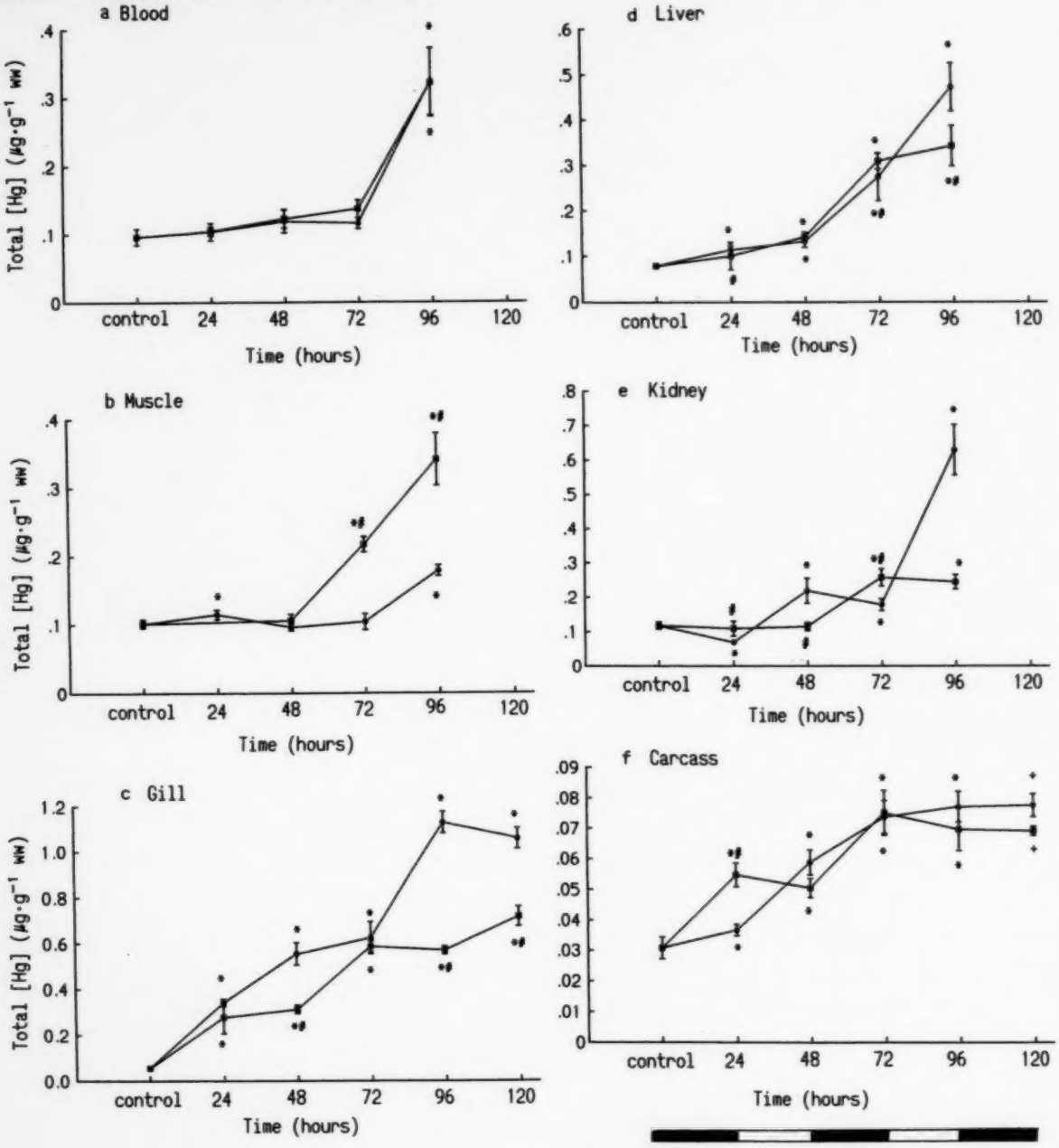
Calculations

The percentage of the whole body mercury contents in each organ for the trout and goldfish was calculated by dividing the mean whole organ metal content by the whole body burden multiplied by 100 percent. Whole body burdens of total mercury were determined by summation of the individual tissue sample contents plus the body carcass for each fish. The average mercury contents of whole organs were determined from tissue metal concentrations multiplied by the relative proportion of body weight for each organ (Gingerich et al. 1987).

Statistics

Data for each species were ranked according to exposure time and regime, then each set of tissue data was tested by analysis of variance (ANOVA, significance level of $P = 0.05$) and multiple range tests using "Statgraf" (Statgraphics Corp., USA).

Fig. 2. Total mercury concentrations in the tissues of rainbow trout during intermittent (■) and continuous (●) exposure to mercuric chloride. Data are means ± SE for seven fish. *Significant difference from control. #Significantly different from continuous exposure at the corresponding exposure time. +Only two observations. Solid bar signifies exposure; open bar represents recovery periods of intermittent exposure.



Results

Trout

The tissues of control trout contained about 0.1 $\mu g \cdot g^{-1}$ wet weight (ww) of mercury or less (Fig. 2). The gills, liver, and kidney contained less than 1% of the body burden, while the blood stored only 2% of the total contamination (Table 1). In contrast, the muscle contained about a quarter of the body burden and at least two thirds of the body burden remained in the carcass.

Table 1. Distribution of mercury in organs of trout and goldfish during intermittent and continuous exposure (%).

		Trout		Goldfish	
Time	Organ	Continuous exposure	Intermittent exposure	Continuous exposure	Intermittent exposure
0 (control)	Blood	2.18	2.18	3.46	3.46
	Muscle	28.2	28.2	53.6	53.6
	Gill	0.87	0.87	1.64	1.64
	Liver	0.44	0.44	1.26	1.26
	Kidney	0.55	0.55	1.25	1.25
24 h	Blood	1.63	1.49	2.49	2.58
	Muscle	22.06	nd	31.99	38.59
	Gill	3.65	2.69	2.66	2.88
	Liver	0.44	0.36	0.71	0.69
	Kidney	0.22	0.32	0.53	0.56
48 h	Blood	1.22	1.41	3.45	3.65
	Muscle	12.04	14.88	31.56	53.97
	Gill	3.85	2.45	3.92	4.48
	Liver	0.34	0.41	0.48	0.98
	Kidney	0.46	0.27	0.4	0.73
72 h	Blood	0.94	1.38	1.8	2.9
	Muscle	10.22	26.83	20.64	37.87
	Gill	3.43	4.01	3.88	4.81
	Liver	0.55	0.77	0.29	0.67
	Kidney	0.29	0.53	0.21	0.37
96 h	Blood	2.494	2.8	3.35	1.89
	Muscle	16.92	36.53	32.81	27.44
	Gill	5.939	3.38	7.59	3.09
	Liver	0.908	0.74	0.58	0.45
	Kidney	0.999	0.44	0.44	0.31
120 h	Blood	nd	nd	1.66	2.34
	Muscle	nd	nd	17.18	25.96
	Gill	5.67	8.35	4.02	4.3
	Liver	nd	nd	0.36	0.56
	Kidney	0.79	0.77	0.25	0.47

Note: Values are mean % of the body burden for six goldfish or seven trout. nd = no data.

Exposure to either continuous or intermittent concentrations of mercury resulted in statistically significant contamination of all the tissues examined compared with the control at time zero (Fig. 2). The gills, liver, and kidney showed the greatest increases in mercury concentrations, reaching maximum values of 1.12 ± 0.04, 0.47 ± 0.05, 0.62 ± 0.07 $\mu g \cdot g^{-1}$ ww, respectively (mean ± SE, $n = 7$) after 120 h of exposure. The blood and muscle were slower to accumulate the metal, reaching concentrations of 0.32 ± 0.04 and 0.34 ± 0.03 $\mu g \cdot g^{-1}$ ww, respectively (mean ± SE, $n = 7$) after 120 h of exposure. About 40% of the body burden was contained in the muscle (Table 1).

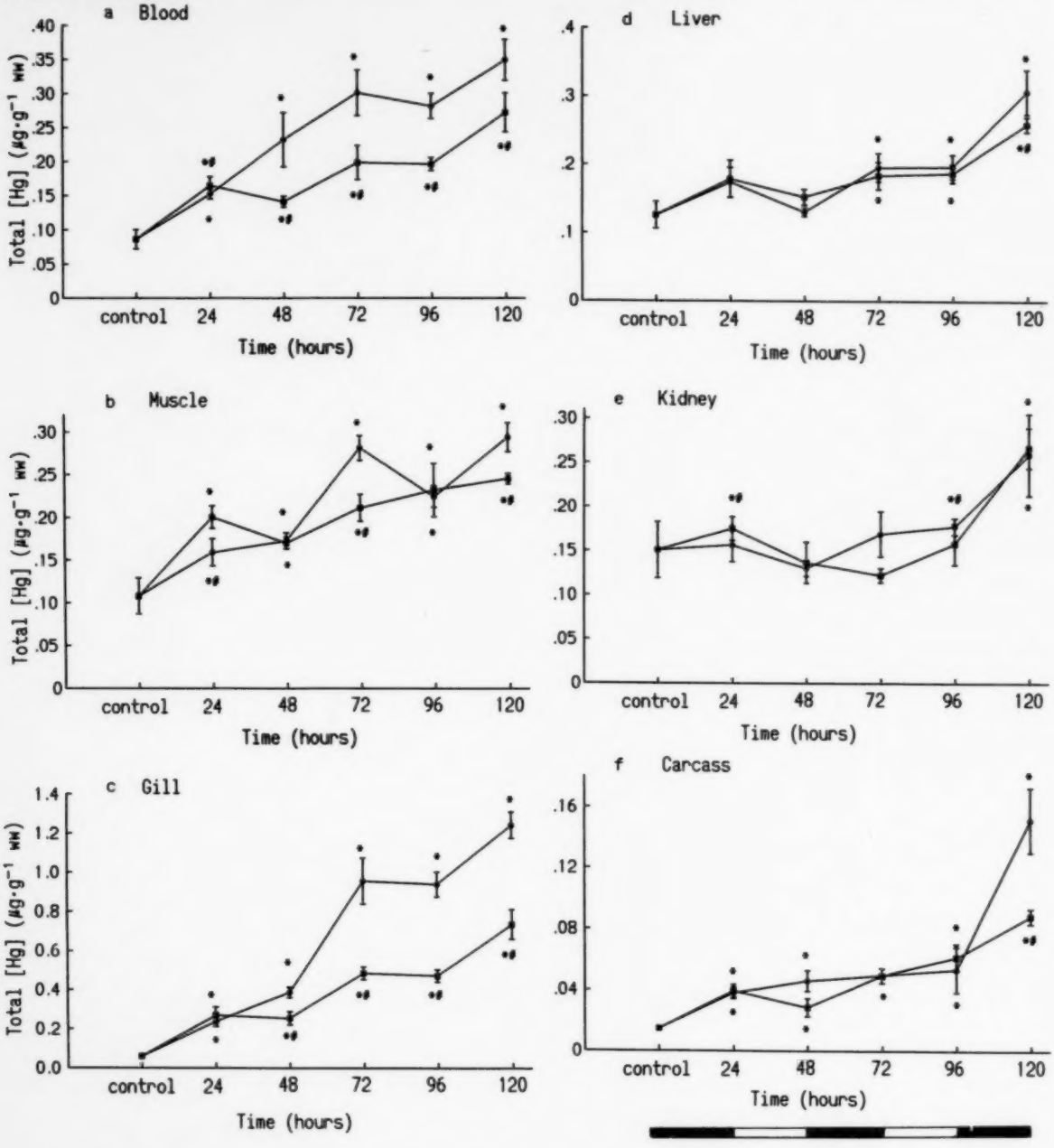
Tissue-specific differences in accumulation also varied with exposure regime. The gills of continuously exposed fish contained significantly more mercury by 120 h (1.05 ± 0.04 $\mu g \cdot g^{-1}$ ww) than those of animals intermittently exposed (0.72 ± 0.04, $n = 7$, mean ± SE). Branchial accumulation was rapid; accumulation occurred in phase with periods of exposure and recovery (Fig. 2*c*). The liver, kidney, and carcass showed a less prominent exposure regime dependent accumulation, but in all cases the tissues of continuously exposed fish contained more mercury than those of intermittently exposed animals at 120 h (Figs. 2*d*, 2*e*, and 2*f*). The blood only showed significant contamination at 96 h with a mean mercury concentration of 0.32 $\mu g \cdot g^{-1}$ in both regimes. Thus no significant difference between exposure profiles was observed (Fig. 2*a*). The muscle of trout showed an unexpected accumulation, with metal concentrations being higher in the intermittently compared with the continuously exposed group (Fig. 2*b*).

The whole body burdens of mercury increased with exposure time in both regimes. The final distribution of the body burden between internal organs was similar in both regimes (Table 1). However, the accumulation during intermittent exposure was more variable with the lowest (14%) and highest (36%) proportions of the body burden appearing in the muscle during recovery periods (Table 1).

Goldfish

The tissues of control goldfish typically contained 0.1 $\mu g \cdot g^{-1}$ ww for mercury or less (Fig. 3). The gill, liver, and kidney of control animals contained a total of 6%,

Fig. 3. Total mercury concentrations in the tissues of goldfish during intermittent (■) and continuous (●) exposure to mercuric chloride. Data are means ± SE for six fish. *Significant difference from control. #Significantly different from continuous exposure at the corresponding exposure time. Solid bar signifies exposure; open bar represents recovery periods of intermittent exposure.



while the blood stored 3.5% of the toxicant body burden. The muscle held 50% of the contamination in each fish.

Waterborne exposure to inorganic mercury contaminated all the tissues examined, regardless of the exposure profile (Fig. 3). The gills displayed the greatest increases in mercury concentration, increasing from a control value of 0.06 $\mu g \cdot g^{-1}$ ww to a maximum of 1.24 $\mu g \cdot g^{-1}$ ww at 120 h during continuous exposure. The blood and liver exhibited a three-fold increase in toxicant concentrations reaching maximums of about 0.34 and 0.30 $\mu g \cdot g^{-1}$ ww,

respectively, by 120 h of continuous exposure. The muscle and carcass also showed progressive contamination with time in both exposure regimes.

The exposure profile had statistically significant effects on mercury accumulation by most of the tissues examined (Fig. 3). Continuous exposure generally produced greater accumulation than intermittent exposure, with the possible exception of the kidney. As in trout, the gills of goldfish showed the clearest differences in concentrations between regimes (Fig. 3*c*). Branchial mercury concentrations were higher during continuous than intermittent exposure, and the gills of intermittently exposed fish accumulated mercury in phase with periods of exposure. The blood showed a similar accumulation pattern with exposure profile (Fig. 3*a*). The liver and carcass exhibited progressive mercury accumulation with time during both exposure regimes, with tissue mercury concentration in continuously exposed fish being significantly higher than in intermittently exposed animals by 120 h (Figs. 3*d* and 3*f*). The kidney showed significant differences between regimes, but no time-dependent trend (Fig. 3*e*). Similarly, muscle toxicant concentrations were significantly higher in continuously exposed animals at 24, 72, and 120 h compared with intermittent values, but an exposure time-dependent difference between exposure profiles was absent (Fig. 3*b*).

The whole body burdens of goldfish increased with exposure time in both regimes and the proportion of mercury stored in the muscle decreased as other organs became contaminated. However, no obvious differences in the distribution of mercury between exposure profiles were observed (Table 1).

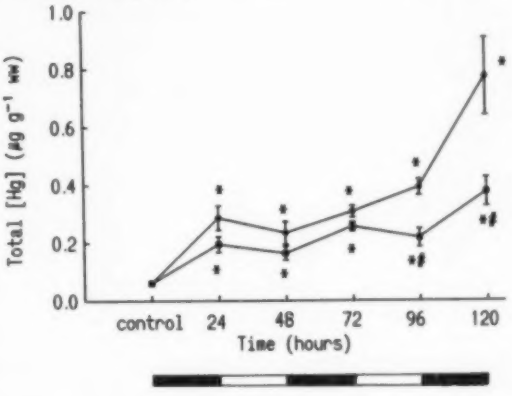
Fathead minnow

The body burden in fathead minnows increased with exposure time in both regimes. The mean body concentrations were generally higher in continuously compared with intermittently exposed fish, with statistically significant differences appearing at 96 h of exposure (Fig. 4). Whole body burdens increases to 0.77 ± 0.13 (continuous) and 0.37 ± 0.05 (intermittent) by 120 h compared with 0.06 ± 0.005 $\mu g \cdot g^{-1}$ ww (mean ± SE, $n = 7$) in control fish. Accumulation and depuration of mercury was in phase with the exposure profile for fathead minnows.

Mortalities and toxicity

No mortalities were observed among the goldfish or trout, but deaths occurred in the minnow experiment. Six fish died during continuous exposure compared with three during intermittent exposure, with body burdens of 0.14 ± 0.03 (mean ± SE, $n = 6$) and 1.03 ± 0.46 ($n = 3$) $\mu g \cdot g^{-1}$ ww, respectively. The body burdens in moribund fish from the continuous exposure regimes were not significantly different from concentrations in surviving fish. Only the three dead intermittently exposed fish had much higher mercury concentrations than surviving minnows. The body weight of dead animals (0.34 g continuous, 0.20 g intermittent exposure) was less than the 0.47-g average for surviving fish, implying a relationship between small body size and mortalities. No mortalities and no other sign of toxicity were observed (loss of equilibrium, swimming ability) in any of the control fish.

Fig. 4. Total mercury concentrations in the bodies of fathead minnows intermittently (■) or continuously (●) exposed to mercuric chloride. Data are means ± SE for seven fish. *Significant difference from control. #Significantly different from continuous exposure at the corresponding exposure time. Solid bar signifies exposure; open bar represents recovery periods of intermittent exposure.



Discussion

Accumulation during continuous exposure

The accumulation of mercury by trout in the continuous exposure regime was similar to previous reports for salmonids (Macleod and Pessah 1973; Olson et al. 1973; McKim et al. 1976; Philips and Buhler 1978; Ramamoorthy and Blumhagen 1984; Barak and Mason 1990; Harrison et al. 1990; Schmitt and Brumbaugh 1990; Bloom 1992). The total mercury concentration in the organs of control trout (0.004–0.117 $\mu g \cdot g^{-1}$ ww, Fig. 2) and the distribution of the body burden were comparable to previous studies with the muscle supporting a quarter of the body burden, while the gills, liver, and kidney contained only a small percentage of the contamination. Similar to McKim et al. (1976), two thirds of the body burden were stored in tissues remaining in the carcass (Table 1). Much of this "missing" contamination could be associated with surface mucus (79% of the body burden in goldfish (McKone et al. 1971)), bone, body fat, or parts of the nervous system not dissected. Mercury was accumulated in or on the gills during continuous waterborne exposure (Fig. 2*c*) and then transferred to the liver, kidney, and muscle as reported in previous studies.

There have been relatively few studies of mercury accumulation by goldfish (McKone et al. 1971; Sharma and Davis 1980*a*, 1980*b*; Weisbart 1973), and only the study of Weisbart (1973) gives a comprehensive account of accumulation, albeit with fish injected with mercury. Thus the data reported here are probably the first detailed report for the goldfish of mercury accumulation from the water. The total mercury concentrations in tissues and the distribution of the body burden in control goldfish (Fig. 3) were similar to the rainbow trout (Fig. 2). About 40% of the body burden was also unaccounted for in the analysis. Mercury accumulation during continuous waterborne exposure

was similar to that previously reported of intraperitoneally injected goldfish. The metal was rapidly accumulated into the blood via the gills. Then the liver, kidney, and muscle became progressively contaminated with exposure time (Fig. 3; Weisbart 1973).

Mercury accumulation and toxicity to the fathead minnow have been studied on several occasions (Olson et al. 1975; Snarki and Olson 1982; Harrison et al. 1990), and as expected, accumulation increased with exposure time in the continuous regime (Fig. 4). The background whole body burden for the fathead minnow (Fig. 4) was lower than that for the other fish used in this study, probably due to the size-dependent accumulation of mercury in fish (Newman and Doubet 1989).

Accumulation during intermittent exposure

The target organs for mercury accumulation were the same in both intermittent and continuous exposure regimes (Figs. 2 and 3). The different exposure regimes also had no discernible effect on the distribution of mercury within contaminated fish (Table 1). Thus the mode of mercury accumulation is unaffected by the exposure profile. However, the absolute concentrations in the tissues were greatly influenced by the type of exposure (Figs. 2, 3, and 4).

Continuous exposure to mercuric chloride produces a broadly predictable pattern of time-dependent accumulation, where toxicant accumulation increases with exposure time until tissue saturation occurs (McKone et al. 1971; Macleod and Pessah 1973; Snarki and Olson 1982). Intermittent exposure also resulted in a time-dependent accumulation where intermittently exposed fish accumulate less mercury than continuously exposed animals (Figs. 2–4). The cause of the lower mercury concentrations in the organs of intermittently exposed fish arose from reduced or negligible accumulation during recovery periods of the regime, rather than excretion of mercury (Figs. 2–4). No excretion of mercury was expected in the recovery periods, because the biological half-life of inorganic mercury is about 3 weeks (Weisbart 1973). In continuous exposure studies mercury accumulation rates are much higher (10–100 times) than the depuration rates (Weisbart 1973; McKim et al. 1976). Consequently recovery must be much longer than the exposure period to produce a decrease in tissue contamination.

The amount of mercury accumulation during intermittent exposure in these experiments was therefore proportional to exposure duration. Hence in the minnow experiment, intermittently exposed fish were expected to contain 40% less mercury than the continuously exposed fish at 120 h; the actual difference was 44% (Fig. 4). The tissues of the intermittently trout and goldfish also accumulated about 60% of the mercury concentrations found in the continuously exposed group, with the possible exception of the trout carcass and goldfish muscle (Figs. 2 and 3).

The temporal effects of multiple exposures on accumulation will only be proportional to the continuous exposure duration when peak concentrations of pollutant are the same in both regimes. Previous studies of intermittent exposure were hampered because higher peak concentration in the intermittent regimes provided greater concentration gradients for diffusive entry of the toxicant compared with the continuous exposure. Thus, results were only comparable using an equivalent mean concentration over a specified time (Seim et al. 1984; Curtis et al. 1989; Siddens et al. 1986). The experiment here have improved on previous protocols by using the same peak concentrations in both regimes. Therefore only the durations of exposure and recovery periods influenced the overall accumulation. However, not all aspects of mercury accumulation were clearly related to the exposure phase. For example, the muscle of goldfish showed a gradual increase in mercury content during the recovery periods of intermittent exposure (Fig. 3*b*). This implies that chelated mercury was being transferred, presumably via the liver, to the muscle for storage during recovery. The blood of the trout also did not give a clear response, presumably because the blood remained saturated with mercury in the recovery periods (Fig. 2*a*).

Toxicity

The acute toxicity of mercuric chloride to fish range between 33 and 687 $\mu g \cdot L^{-1}$ (96 h LC_{50} (Spry and Wiener 1991)). The 5-day LC_{50} for the fathead minnow is about 112 $\mu g \cdot L^{-1}$ (Snarki and Olson 1982), while the median tolerance limit for rainbow trout ranges between 220 and 400 $\mu g \cdot g^{-1}$ (Macleod and Pessah 1973). Thus no mortalities were expected in this study and none occurred in the trout or goldfish experiments. However, cumulative mortalities of 14.2 and 7.1% for continuous and intermittent exposure were observed in the fathead minnow experiment. The fatalities are related to the small size of the dead fish compared with the surviving animals, but also to the exposure regime. More fish died during the continuous than intermittent exposure. Similar observations have been made for H^+ and Cd (Curtis et al. 1989; Handy 1992), but the reverse is true for Cu, which is more toxic during intermittent exposure (Seim et al. 1984; Handy 1992).

In conclusion, intermittent exposure to mercuric chloride does not affect the target organs, or the distribution of the body burden in fish. When peak concentrations of mercury are identical in different exposure regimes, tissue concentrations of mercury will be influenced by the number of repeated short exposures. The tissue concentrations will be proportional to exposure time and may therefore be predicted from continuous exposure data. The environmental agencies could therefore predict accumulation before consent to discharge a variable mercury-containing effluent is granted.

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Path analysis of collective properties and habitat relationships of fish assemblages in coastal plain streams

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Abstract: Collective properties of fish assemblages at 44 sites in low-gradient streams (orders 1–3) on the upper coastal plain of South Carolina, U.S.A., were highly variable among sites: richness, 2–17 species per site; density, 0.2–9.5 individuals·m^{-2}; mean individual mass, 0.4–22.2 g per individual; and biomass, 0.1–42.1 g·m^{-2}. Principal components analysis reduced 15 habitat parameters to four interpretable components that were used as orthogonal predictive variables in path analysis. Path analysis, with a directional model containing intermediate steps and multiple dependent variables, was used as an alternative to multiple regression. Two principal components, associated with velocity (and its covariates) and depth (and its covariates), were the primary correlates of biomass (R^2 = 49%), richness (44%), mean size (36%), and density (20%), with cover variables contributing relatively little to any R^2 value. Velocity effects were negative and depth effects positive for all collective properties. Habitat effects on biomass were mediated by effects on individual fish size rather than numbers. Correlations of collective properties at sites sampled in 2 years were similar to those from the single year, habitat-based path model, implying that the path model was adequate and inclusive, and accurately reflected collective properties of these communities.

Résumé : Les propriétés collectives des assemblages de poissons, observées à 44 sites dans des cours d'eau à faible gradient (ordres 1–3), dans la partie supérieure de la plaine côtière de Caroline du Sud (États-Unis), étaient extrêmement variables d'un site à l'autre : richesse, 2–17 espèces par site; densité, 0,2–9,5 individus·m^{-2}; masse moyenne individuelle, 0,4–22,2 g par individu; biomasse, 0,1–44,1 g·m^{-2}. L'analyse des composantes principales a réduit les 15 paramètres de l'habitat à quatre composantes interprétables qui ont servi de variables prédictives orthogonales dans l'analyse causale. L'analyse causale, avec un modèle directionnel comportant des étapes intermédiaires et des variables dépendantes multiples, a été utilisé à la place de la régression multiple. Deux composantes principales, associées à la vitesse (et ses covariables) et à la profondeur (et ses covariables) constituaient les premiers corrélats de la biomasse (R^2 = 49%), de la richesse (44%), de la taille moyenne (36%) et de la densité (20%), tandis que les variables associées à la couverture contribuaient relativement peu aux valeurs de R^2. Les effets de la vitesse étaient négatifs, et ceux de la profondeur positifs pour toutes les propriétés collectives. Les effets de l'habitat sur la biomasse se traduisaient par des effets sur la taille individuelle des poissons plutôt que sur les effectifs. Les corrélations des propriétés collectives aux sites échantillonnés pendant 2 ans étaient similaires à celles du modèle causal fondé sur l'habitat, couvrant une seule année, ce qui permet de penser que ce modèle était satisfaisant et exhaustif, et représentait de façon exacte les propriétés collectives de ces communautés.

[Traduit par la Rédaction]

Introduction

To better understand ecological communities, patterns may be usefully examined across spatial and temporal scales and with differing aggregations of component taxa (Orians 1980; O'Neill et al. 1986; Allen and Hoekstra 1992). One approach to understanding communities is the description of aggregate, taxon-free properties that are usually expressed in units of mass, energy, or diversity. Analysis of such "collective properties" may detect patterns not apparent at the species level and can provide taxonomy-free comparisons of communities in differing environments (e.g., Kelso and Johnson 1991) or with independent evolutionary histories (e.g., Mahon 1984; Mahon and Balon 1985). The stream environment, structured by hydraulic forces and geology, offers relatively similar systems replicated

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throughout the world, affording the opportunity to detect general patterns of community properties. Furthermore, a growing interest in collective properties and aggregated systems models for management of multispecies commercial fisheries (Regier and Henderson 1973; Dickie and Kerr 1982; Sainsbury 1988; Kerr and Ryder 1989) suggests extension and eventual application of collective property concepts.

In this paper we relate four collective or aggregated measures of stream fishes in a moderately diverse fauna to spatial variation in habitat structure, and examine interrelationships of the collective variables themselves. This analysis of mean individual mass, numerical density, biomass density, and species richness and diversity complements and extends our previous, species-focused descriptions of the same system (Meffe and Sheldon 1988, 1990; Sheldon and Meffe 1993).

Methods

We collected fishes and measured habitat variables (Meffe and Sheldon 1988) at 44 sites in first to third order streams on the U.S. Department of Energy's Savannah River Site (SRS), located on the upper coastal plain in Aiken and Barnwell counties, South Carolina. Waters of the area are soft (1.4–75 $\mu mos \cdot cm^{-1}$), moderately acidic (pH 4.6–8.1, usually <7.0), and clear but colored by organic compounds. Low-gradient (0.2–3 $m \cdot km^{-1}$) streams flow at low to moderate velocity in sandy to muddy channels bordered and shaded by cypress (*Taxodium distichum*) and bottomland hardwood forests. Abundant woody material in the streams controls channel form and provides cover for fish. Approximately 80 fish species are known from the streams, swamps, and ponds of the SRS and the bordering Savannah River (Bennett and McFarlane 1983).

Collections were made 15 October – 19 November, 1985 when young-of-the-year fish were large enough to be identified easily, and flows were relatively stable. Fish were still feeding on abundant terrestrial arthropods (Sheldon and Meffe 1993) so we consider habit occupancy representative of the late summer–fall period rather than a winter retreat to deeper water or downstream reaches. We also sampled 37 of these sites 1 year later using identical methods Meffe and Sheldon (1990). In this paper we compare the collective properties at these sites between years but do not conduct a second path analysis of habitat – collective property relationships.

Sample sites (4–15 m long, $n = 44$) were selected visually to be representative of homogeneous single habitats (e.g., pool, sandy run) and to cover the range of habitats available at 12 major collecting locations on six different streams or tributaries. Sites were isolated with 6-mm mesh block seines and electrofished 5–7 times with 500–700 V alternating current. Final runs captured few or no fish, indicating near-complete capture. Preserved collections (10% Formalin) were sorted, measured, and weighed in the laboratory. Habitat variables (depth, width, velocity, substrate) were measured on a grid system, and cover components (logs, undercuts, brush, leaf packs) were mapped to scale and measured with a planimeter. Meffe and Sheldon (1988) provide further methodological details.

For this analysis, and for a species-specific description of habitat use (Meffe and Sheldon 1988), we objectively reduced by principal components analysis (PCA, Gauch 1982) the 15 habitat variables to a small set of uncorrelated variables. We used these derived variables (principal component scores) in place of the original measures. The PCA was done with the correlation matrix of transformed (logarithms or arcsine square root) data using SYSTAT (Wilkinson 1987). Meffe and Sheldon (1988) further discuss the transformations and the PCA.

Relationships of the collective biological properties to habitat structure were evaluated by path analysis (Sokal and Rohlf 1981). Path analysis, developed by Wright (1921), uses the arithmetic of linear multiple and partial regression but differs from regression in its imposition of a directional or hypothesized cause-and-effect model on the entire variable set. Path analysis does not conform or prove causal sequences; it merely allows one to evaluate a working model, expressed as a path diagram, defined by logic and external evidence concerning mechanisms. Complicated relationships can sometimes be expressed in several alternative and reasonable path diagrams (e.g., Shively and Jackson 1985; Redmond 1986). Choice among models depends not only on the variance accounted for (R^2) but also on the logic of construction and compatibility with known or hypothesized mechanisms. Biddle and Marlin (1987) and Mitchell (1992) discuss evaluation of structural models.

Because path analysis rests on the variance-covariance structure of the data, one should ask if the more familiar multiple regression analysis would be as good or better. Multiple regression yields an equation that retains the original units of measure and allows quantitative prediction of the single dependent variable. Independent variables appear as a set in which possible directional interactions are not shown. Alternatively, path analysis uses standardized coefficients, so units are lost. Path analysis does allow predictions of direction and relative magnitude of effects, and includes the need to formulate (diagram) a problem more explicitly. Path analysis can accommodate a series of intermediate steps and several criterion (dependent) variables in a single analysis (e.g., Arnold 1972; Schemske and Horvitz 1988; Fjeld and Rognerud 1993). One can also evaluate models (hypotheses) in which some paths are assumed unimportant and coefficients are set to zero (Mitchell 1992).

Our path model, illustrated in results, was the only model tested. Although we drew and considered structurally different path models, we did not do a step-wise search for a best model. We assumed that habitat features were primary, and examined consequences of that assumption. Procedures followed Sokal and Rohlf (1981) and were executed with least-squares routines in SYSTAT (Wilkinson 1987). We used coefficients of determination (R^2) and path coefficients (standard partial regression coefficients) to evaluate the path model (Biddle and Marlin 1987).

Results

We collected 30 species of fish in 11 families (Meffe and Sheldon 1988). Minnows (Cyprinidae) dominated numerically, but biomass was more equitably distributed among

Table 1. Species diversity and relative numerical and biomass abundance of fishes collected, by family ("+" = fraction of a percent).

Family	Species	Numbers (%)	Biomass (%)
Anguilidae	1	2	19
Umbridae	1	+	+
Esocidae	2	+	3
Cyprinidae	6	56	20
Catostomidae	3	3	8
Ictaluridae	5	7	17
Aphredoderidae	1	11	10
Cyprinodontidae	1	+	+
Poeciliidae	1	3	+
Centrarchidae	6	13	21
Percidae	3	5	2
Total	30	100	100

families (Table 1). The median sample contained 7 species (S) (range 2–17) at a density ($\overline{N}$) of 1.5 individuals·m^{-2} (0.2–9.5), an individual mass ($\overline{W}$) of 2.8 g (0.4–22.2) and a biomass (B) of 4.0 g·m^{-2} (0.1–42.1). The corresponding means were S = 8.0, $\overline{N}$ = 1.9, $\overline{W}$ = 4.2 and B = 8.2.

The four largest principal components (PC I–IV), all with eigenvalues >1, accounted for 59% of the variance in the 15 habitat variables. Table 2 summarizes the contribution of the original variables to each PC. Principle component I (20% of total variance) is readily interpreted as a gradient from sites with higher current velocity and sandy substrate to units with low velocity and silty or leafy substrate. PC II (17%) separates deep, wide, muddy sites from shallow and narrow sites with other substrates. PC III (11%) separates the rare sites with plant beds from those with gravel substrate and PC IV (11%) loads most heavily on structural variables (leaf packs, wood, roots, and undercut banks). Higher components are small and less interpretable, so are not presented.

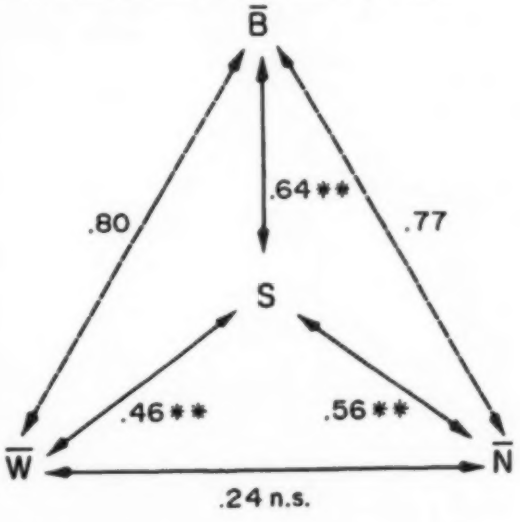
Interrelationships of collective biological properties were evaluated by product-moment correlations (Fig. 1). Because the distributions of collective properties were strongly skewed, they were transformed to logarithms to achieve linearity and homogeneity of variance. Transformation also converted B from the product of $\overline{N}$ and $\overline{W}$ to log B, the additive combination of log $\overline{N}$ and log $\overline{W}$; additivity greatly simplifies the construction of a path model. Correlations are shown as double-headed arrows because there is no clear sequence of cause and effect. One could, for example, suppose the biomass and species richness correlation (Fig. 1) results from the more efficient use of resources by more diverse assemblages or, alternatively, that high standing crop permits higher richness, which is consistent with the productivity–diversity hypothesis (Pianka 1966). Although several are significant, correlations among the collective properties are small enough to justify the use of $\overline{W}$, $\overline{N}$, and S as minimally redundant descriptors of stream fish assemblages.

Biological properties were also correlated with habitat variables (representative examples in Fig. 2). For example, larger fish inhabited slower sites on PC I (Fig. 2B),

Table 2. Loadings of habitat parameters on the first four principal components. For clarity, we list only those loadings greater than 0.30.

	I	II	III	IV
Mean velocity	0.896			
% silt	−0.842		−0.341	
% leaves	−0.678			
% sand	0.655		−0.468	
Mean depth		0.924		
Mean width		0.766		
% mud		0.716		
% undercuts		0.511		−0.328
% gravel			0.796	
% plants			−0.563	
% leaf packs				0.704
% wood				0.641
% coarse roots	−0.454			−0.583
% fine roots				−0.378
% brush		0.498		
% variance explained	20.0	17.4	11.4	11.0

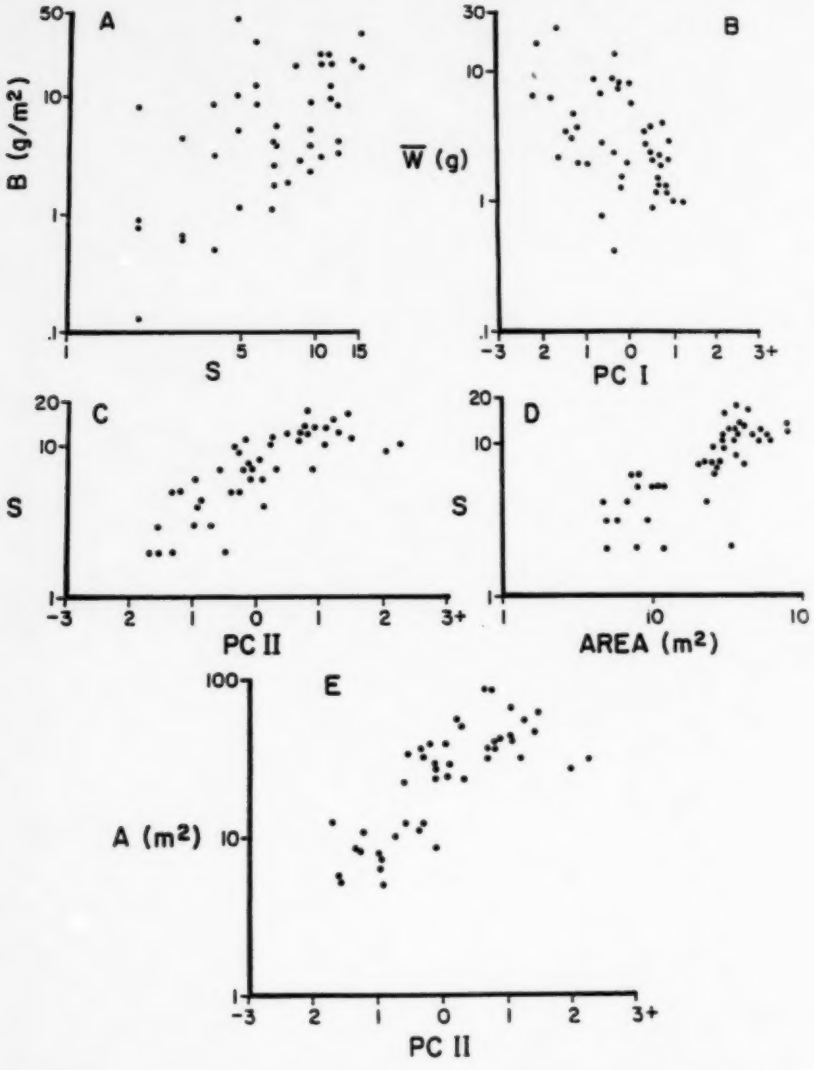
Fig. 1. Correlation plexus of log-transformed collective variables S (species richness), B (biomass), $\overline{W}$ (mean individual weight), and $\overline{N}$ (density). Dashed lines indicate the correlation of B with $\overline{W}$ and $\overline{N}$, from which it is derived and therefore statistical significance is inappropriate. Double asterisks indicate $p < 0.01$.



and deeper locations on PC II had more species (Fig. 2C). However, univariate correlations in a multivariate world can be misleading, as richness was also dependent on total area (A) of the collection site (Fig. 2D), and area and PC II also were correlated (Fig. 2E). This raises the possibility that the S × PC II correlation is an artifact resulting from the correlation of area and the habitat PC.

Species-area dependencies are well known (Connor and McCoy 1979) and can result from greater heterogeneity

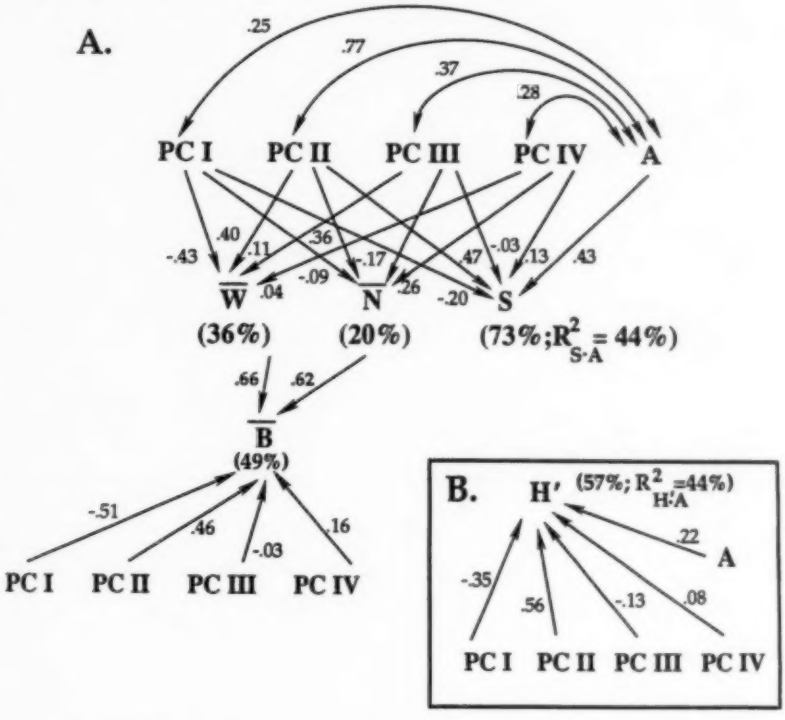
Fig. 2. Relations of representative pairs of biological and habitat variables. A, Biomass (B) and species richness (S); $R^2 = 0.41$. B, Mean weight ($\overline{W}$) and PC I (velocity and covariates); $R^2 = 0.23$. C, Species richness and PC II (depth and covariates); $R^2 = 0.62$. D, Species richness and sample area (A); $R^2 = 0.58$. E, Sample area and PC II, $R^2 = 0.60$.



of larger areas (Williamson 1988) or simple dependence of the number of species observed on the number of individuals examined (Angermeier and Schlosser 1989). The correlation of area and PC II is easily explained: higher order streams tend to be both wider and deeper, so depth and width load heavily on PC II (Table 2). Moreover, our deepest sample sites were entire pools bounded by shallows, features that are typically 5–7 stream widths apart (Leopold et al. 1964). Because deeper sites were both longer and wider, in the following path analysis we treat area (transformed to log A) as a covariate whose effects are to be controlled and removed. This standardizes S, as was done initially for $\overline{N}$ and B. The more complex procedure is required because untransformed S and A are not linearly related.

In the path diagram (Fig. 3A) we assume that density ($\overline{N}$) and individual size ($\overline{W}$) depend directly on the orthogonal variables (PC I–IV). Biomass (B), which is a derived quantity, appears at the end of a path with its components ($\overline{W}$, $\overline{N}$) as intermediate steps. Richness (S) depends on PC I–IV and area. In the notation of path analysis (Sokal and Rohlf 1981), causal pathways are shown as single-headed arrows. Path coefficients on such arrows are standard partial regression coefficients that show the effect of

Fig. 3. Path diagrams showing effects of habitat variables (PC I–IV) and sample area (A) on collective variables $\overline{W}$ (mean weight), $\overline{N}$ (density), S (richness), B (biomass), and H′ (diversity). Path coefficients are shown on single-headed arrows. Double-headed arrows and their correlation coefficients indicate nondirectional effects. Coefficients of determination (R^2, as %) are given next to each collective variable, and partial coefficients of determination ($R^2_{\cdot A}$, with A held constant) are near richness and diversity variables. A, The complete path diagram. The lower left shows the composite paths to B with intervening steps and coefficients condensed. B, An alternative path analysis with H′ substituted for S. Correlations of A with the PC equal those given above.



a predictor variable (with other variables held constant) on the dependent or criterion variable. Ratios of coefficients measure the relative importance of variables as predictors of the dependent variable. The total effect along all pathways, including intermediate steps, is given by the R^2 shown near each dependent variable, and represents the percentage of variation in that parameter explained by the model. Double-headed arrows denote nondirectional bivariate correlations used in computing the path coefficients.

All the collective biological properties were related to habitat attributes. Mean size ($\overline{W}$) was equivalently related to PC I (velocity and covariates) and PC II (depth, width, and covariates) and negligibly to PC III and PC IV; R^2 (36%) was modest. Density ($\overline{N}$) was less strongly determined (R^2 = 20%), with PC II and PC IV (cover) contributing most. Habitat effects on biomass (B), a composite variable, are presented in two ways. The summary pathway from the PCs to B shows that B was equivalently and quite strongly (R^2 = 49%) related to PC I and PC II, with minor contributions from PC III and PC IV. However, examination of the complete pathway from the PC through $\overline{W}$ and $\overline{N}$ shows that habitat effects on biomass were mediated more strongly through individual size (R^2 = 36%) than by density (R^2 = 20%), and the effect of PC I on B resulted solely from its influence on $\overline{W}$ (−0.43) rather than on $\overline{N}$ (−0.09).

Species richness (S) presents a more difficult problem. As noted above, area (A) was included as a predictor variable. When this was done, S was strongly determined (R^2 = 73%), with equivalent contributions from PC II (0.47) and area (0.43). However, if area produced its effects only through its influence on collection size, rather than greater heterogeneity of larger areas, then the partial coefficient of determination ($R^2_{S \cdot A}$ = 44%) is a conservative measure of habitats effects. Partialling out the effect of area probably underestimates habitat influences because A, highly correlated with PC II, is a co-linear and redundant measure of habitat structure.

As a measurement of diversity, we prefer the simple and explicit parameter S (Hurlbert 1971) to more complex and less intuitive diversity indices (e.g., Sheldon 1968; Foltz 1982). However, measures such as the information theoretic H′ (Pielou 1975) are less dependent on sample size

Table 3. Coefficients of determination (R^2) of collective properties from the habitat-based, single year path analysis (Fig. 3, $n = 44$), and between-year collective properties at sites defaunated in this study, recolonized, and sampled 1 year later (Meffe and Sheldon 1990, Fig. 2, $n = 37$).

Collective property	R^2 from path analysis	R^2 for between-year samples
$\overline{N}$	20%	26%
$\overline{W}$	36%	40%
B	49%	51%
S	73%	68%

than is species richness (Wilhm 1972) so we repeated the path analysis with the diversity index H′ as the criterion variable. This procedure increased coefficients of the habitat variables and diminished that of area (Fig. 3B). Although the coefficient of determination was reduced from 73 to 57%, the partial $R^2_{H \cdot A}$ equals $R^2_{S \cdot A}$. Thus, richness–diversity is the most predictable of the primary collective variables and, if allowance is made for co-linearity of area with PC II, is nearly as strongly determined as biomass.

It is natural to suspect that inclusion of additional variables in a model will improve it. The following evidence suggests that our path model is inclusive and adequate. We sampled 37 of the 44 sites that were defaunated in this study 1 year later and demonstrated recovery through recolonization (Meffe and Sheldon 1990). None of the collective properties differed between years (paired *t*-tests), and between-year correlations of collective properties at these sites were significant (Meffe and Sheldon 1990). Virtual identity of coefficients of determination of the single year, habitat-based path analysis, and between-year comparisons of the collective variables themselves (Table 3) demonstrate the adequacy of principal components I–IV as habitat descriptors. In other words, the colonizing fish could not reconstruct the pattern of collective variables any more precisely than described by the path model. The residual variance is a true error term and there appear to be no major unmeasured independent correlates of the collective properties, nor do nonlinear effects appear to be large.

Discussion

Our path analysis (Fig. 3) supports (does not refute) the hypothesis that habitat structure influences collective properties of stream fish assemblages. Reduction of the stream habitat to two primary variables, velocity and channel size and shape (Table 2), is a geomorphologically realistic (Leopold et al. 1964; Richards 1982) but minimalist description (Slobodkin 1986) that provides a satisfactory template for the distribution of species and intraspecific size classes (Meffe and Sheldon 1988) and, as shown here, the collective properties as well.

The path analysis consistently identified PC II (depth and covariates) as an important influence on the collective biological properties. Velocity and its covariates (PC I) also were influential, but the structural or cover components summarized in PC III and PC IV had little effect. In sum, deeper (PC II positive) sites had more species, larger individuals, and slightly larger numbers of fish than did shallow habitats. Fast, sandy sites (PC I positive) had fewer species and smaller individuals than did slow, silty areas.

The effects identified in Fig. 3 are mechanically reasonable and consistent with other studies on stream fishes where depth and covariates (our PC II) and velocity and covariates (our PC I) are frequently identified as controlling factors. Depth preferences influence habitat selection in the horizontal plane (Moyle and Vondracek 1985) and the vertical dimension is partitioned coarsely in benthic and water column guilds (Grossman and Freeman 1987) or more finely by species and size classes (Baker and Ross 1981; Gorman 1987, 1988*a*, 1988*b*). Velocity affects the distribution of species and size classes (Grossman and Freeman 1987; Wikramanayake and Moyle 1989), and diversity, size, and biomass of stream fishes are positively correlated with depth (Sheldon 1968; Mahon and Portt 1985; Portt et al. 1986; Capone and Kushlan 1991; Harvey and Stewart 1991).

Cover and substrate effects appear to be much weaker than those of depth and velocity. In part this occurs because woody material and substrate interact with channel form and flow, thus appearing in PC I and PC II associated with velocity and depth. PC III and PC IV incorporate substrate and cover information independent of depth and velocity, and they influence density slightly, perhaps by providing escape cover for small fish. Experimental results (Angermeier and Karr 1984), changes in fish populations of channelized streams (Tarplee et al. 1971), and high invertebrate production on snags (Benke et al. 1985) imply a major role for woody material. Our results do not contradict this conclusion but do show the difficulty of separating hydraulic and channel-forming effects from those directly and independently influencing fish behavior.

The R^2 values we report, while low, may be as high as could be expected in streams of similar climate and nutrient status and with a limited range of structural and hydraulic characteristics (Brussock et al. 1985). Our results, with those of studies cited elsewhere in the discussion, suggest that collective properties of aquatic systems are intrinsically highly variable. However, Regier (1977) proposes that relationships with this precision are useful in management.

Stream fish assemblages are not constant (Orth and Maughan 1984; Schlosser 1987*a*, 1987*b*; Matthews 1990) so our results must be viewed as a snapshot. However, Matthews (1990) concluded that spatial variability was greater than temporal, at least in summer–early fall, and Orth and Maughan (1984) showed that fall biomass approximated

the annual mean in some stream habitats. Further, our analysis emphasizes direction and relative magnitude of effects rather than absolute quantities, so our conclusions should be reasonably robust to temporal influences.

The path model couples habitat structure directly with aggregated biological responses and ignores mediating mechanisms such as habitat selection and species interactions, including predation. Predation is known to affect movement, size-specific habitat selection, and density (Fraser and Emmons 1984; Bowlby and Roff 1986*a*; Power 1987; Schlosser 1987*a*; Harvey and Stewart 1991). If larger fish, especially piscivores, are limited to deeper, slower sites (Meffe and Sheldon 1988), predation or avoidance by small fish will reinforce the tendency toward greater $\overline{W}$ in such habitats. However, the response is still habitat driven.

Collective properties vary along environmental gradients other than those measured here. For example, extensive geomorphic attributes (e.g., elevation, stream order) have considerable predictive power (Barila et al. 1981; Foltz 1982; Watson and Balon 1984; Lanka et al. 1987), although they cannot be perceived directly by fish. More proximate factors such as heterogeneity measures (Gorman 1978) and food availability (Bowlby and Roff 1986*b*; Racocinski 1988) also can strengthen stream models. Edaphic factors (Thompson and Hunt 1930) could be important in extending our analysis beyond a homogeneous region. Regression and correlation analyses have related productivity, biomass, and diversity in lakes to physical and chemical factors (Ryder 1965; Patalas 1975; Hanson and Peters 1984; Yan 1986). Inclusion of primary production or macrobenthos biomass has strengthened fish production models (Hanson and Leggett 1982; Downing et al. 1990). (Such environmental-trophic linkages could be modeled appropriately by path analysis.) Patterns of collective properties in the sea (e.g., Polloni et al. 1979; Rex 1981; MacPherson and Duarte 1991; Heip et al. 1992) provide parallels with freshwater systems and a message that, as in our data, collective properties have high residual variances, even when clear trends are evident (Lampitt et al. 1986).

Are collective properties meaningful and useful? They are both, if they show resilience under stress or exploitation, and convergence in systems with different evolutionary histories. Evidence for resilience includes stability of yield from a commercial fishery in spite of fluctuations of component species (Sutcliffe et al. 1977). Persson (1986) described an almost perfect compensatory response of perch biomass to reduction of competing roach, and Tonn and Pazkowski (1986) observed a 14-fold increase in mudminnows and restoration of total biomass after winterkill of predatory and competitive perch. Alternatively, displacement of collective properties from regional or habitat-specific norms can be used as a measure of environmental stress. The index of biotic integrity (Karr 1981; Fausch et al. 1984) includes diversity and density components, two collective properties. Effective manipulations (species removal) in open stream systems are difficult (Meffe and Sheldon 1990; Peterson and Bayley 1993; but see Harvey and Stewart 1991) but also provide valuable evidence for resilience and stability of collective properties (Table 3).

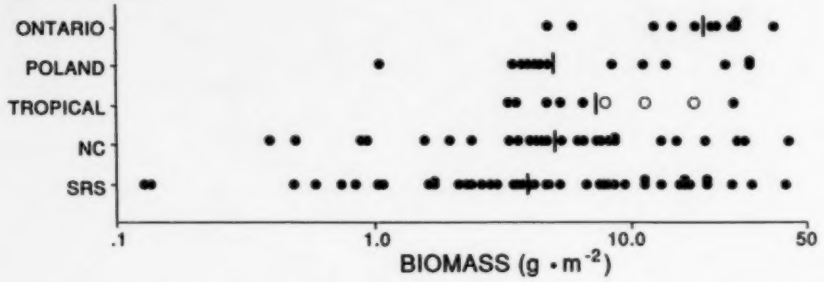
Critics of aggregation approaches (e.g., collective variables, trophic categories) argue validly against the loss of resolution and mechanistic detail entailed by aggregation and censoring species identities. We proposed, however, that analysis of collective properties might reveal patterns undetectable at the species level or permit comparisons where taxonomic composition differed between systems being compared. Wiegert (1988) resolved this issue, in our view, by separating pattern and explanation. He stated that when patterns are detected at one scale or degree of aggregation, explanations are to be found at scales larger or smaller than the phenomenon under consideration but not at the scale of the pattern itself. Thus, patterns of collective properties in single pools and riffles may have explanations in individual behavior, predator–prey interactions, geometry of nearby stream reaches, soil chemistry in the catchment, or location in a major zoogeographic region.

Similarly, definition of collective properties follows from small- or large-scale thinking. We used the term collective properties following Salt (1979), who parsimoniously defined them as sums of single species (and, by implication, individual) values. An alternative perspective is Brown's (1981, 1987) distinction between "capacity rules" and "allocation rules," in which system capacity, expressed as collective variables, is determined by extrinsic environmental factors, primarily energetic ones in Brown's view. Allocation, division of system capacity, is seen as a secondary process regulated by biotic interactions and the adaptive repertoires of species. We followed Salt's (1979) arithmetic view of collective variables and, in so doing, avoided the term "emergent property" (Edson et al. 1981). However, our emphasis on environmental control is logically similar to Brown's (1981, 1987) capacity concept.

Our path analysis also suggests hypotheses and explanations from above and below the level of collective properties. For example, biomass could be a function of energy availability. This hypothesis could be tested only by comparisons with other systems differing in nutrient status or productivity. Alternatively, because habitat influences biomass through mean size (Fig. 3), we hypothesize that biomass depends on habitat selection or differential mortality of individuals. Experiments by Harvey and Stewart (1991) support this view. They dug 16 pools in a small stream. Three weeks later the size of the largest colonizing fish in each pool was significantly greater in deep pools than in shallow ones. Their experimental results are consistent with our data because individual size and biomass differed between treatments but numerical density, although greater in deep pools, was not significantly so. If fish are as mobile and selective as implied by these (Harvey and Stewart 1991) and other results (Meffe and Sheldon 1990; Peterson and Bayley 1993; Bayley and Osborne 1993), the distribution of collective properties among stream segments may result from the matching of individual fish to habitat, plus a neighborhood effect (Osborne and Wiley 1992) and larger scale patterns driven by habitat gradients and ontogenetic habitat shifts (Schlosser 1987*b*).

Independent evolution of fish faunas on different continents (Moyle and Cech 1982) and in different drainages within continents (Hocutt and Wiley 1986) also offer significant opportunities for ecological comparisons and generalization. The few such comparisons (Mahon 1984; Watson and Balon 1984; Mahon and Balon 1985; Moyle and

Fig. 4. Biomass of stream fishes from five geographic regions, with median values indicated by vertical lines. Sources are Ontario and Poland (Mahon and Balon 1985), tropics (Borneo (●) (Watson and Balon 1984), Thailand (○) (Geisler et al. 1979)), North Carolina (unmodified streams only) (Tarplee et al. 1971), and Savannah River Site (this study).



Herbold 1987; Tonn et al. 1990) indicate convergence of some aspects of community structure but not of others. Both historical (Mayden 1987) and ecological factors operating at various scales (Tonn et al. 1990) determine geographical variation in assemblages of fishes. Biomass data from three continents, and latitudes from equatorial to 50°N (Fig. 4), illustrate the difficulties of such comparisons. Such data are extremely variable, and a visual comparison of medians and ranges suggests little difference between regions. In particular, our data correspond with those of Tarplee et al. (1971) from similar coastal plain streams with many shared species. Mahon and Balon (1985) concluded that Ontario streams supported higher biomass than did Polish streams. However, the Ontario data are notably lacking in low values while maxima are not very different. As ~50% of the variance in our data can be attributed to differences in depth and velocity (Fig. 3), habitat measurements and covariate control are obviously necessary for more detailed comparisons. (Similarly, Paloheimo and Regier (1982) argue that environmental variables must be included in yield models.) Not only could means be compared better, but the structure of path diagrams relating habitat to the entire set of collective variables could be compared.

Collective properties of diversity, size, density, and biomass are useful comparative and summary statistics for fish assemblages. Simultaneous consideration of these measures by path analysis suggests mechanisms that regulate them in natural streams. The path model also suggests the changes to be expected in physically modified streams (Tarplee et al. 1971; Shields et al. 1994).

Collective properties, at the scale reported here, are midway in a hierarchy of scales and mechanisms. Path analysis and related structural models could be powerful tools for understanding the entire hierarchy. Experimental, as well as observational data can be incorporated into path models (Mitchell-Olds 1987), and the structure of path models can suggest critical experiments to dissect out the major environmental and biological factors affecting community structure.

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Trout population response to habitat enhancement in six northern Colorado streams

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Abstract: We examined the effects of log drop structures on trout populations in six small, remote Rocky Mountain streams. Angling pressure was low on all streams, and most anglers killed no fish. Log drop structures were installed in 250-m treatment sections in summer 1988, and results were compared with adjacent 250-m control sections during 1987–1990. The structures caused marked changes in habitat, including greatly increased pool volume, decreased current velocity, and increased depth and cover. After the structures were installed, abundance and biomass of age-2 and older trout (and often age-1 trout) increased in all six streams, but there was no evidence that trout were in better condition or grew to larger sizes in most streams. Recaptures of tagged trout in two streams showed that the logs did not result in increased growth or survival of resident trout, although recaptures of fin-clipped trout in other streams suggested that apparent survival may have increased temporarily in treatment sections. Low recapture rates of marked trout and high percentages of unmarked adults indicated that immigration rates were high, suggesting that movement is an important mechanism to account for the increase in trout populations after habitat enhancement in these streams.

Résumé : Nous avons examiné les effets de l'installation de déversoirs en bois sur les populations de truites de six petites ruisseaux peu accessibles des Montagnes Rocheuses. La pression de pêche sportive était faible sur tous ces cours d'eau, et la plupart des pêcheurs ne tuaient aucun poisson. Les structures ont été installées dans des tronçons aménagés de 250 m à l'été 1988, et les résultats ont été comparés à ceux de tronçons témoins adjacents, de 250 m, entre 1987 et 1990. Les déversoirs ont causé des modifications marquées de l'habitat, notamment une forte augmentation du volume des fosses, une baisse de la vitesse du courant, ainsi qu'un accroissement de la profondeur et de la couverture. Après l'installation des structures, l'abondance et la biomasse des truites de deux ans et plus (et souvent d'un an) a augmenté dans les six cours d'eau, mais rien n'indiquait que les truites étaient en meilleure santé ou atteignaient des tailles plus élevées dans la plupart des ruisseaux. La recapture dans deux ruisseaux de truites marquées a révélé que l'installation des déversoirs ne causait pas d'augmentation de la croissance ni de la survie des truites résidantes, mais la recapture dans les autres cours d'eau de truites aux nageoires coupées semble indiquer que le taux apparent de survie peut avoir augmenté temporairement dans les tronçons aménagés. Le faible taux de recapture de truites marquées et le fort pourcentage d'adultes non marqués indiquent que le taux d'immigration était fort, ce qui permet de penser que le déplacement constitue un mécanisme important qui explique l'accroissement des populations de truite après l'aménagement de l'habitat dans ces cours d'eau.

[Traduit par la Rédaction]

Introduction

The importance of habitat to stream salmonid populations is generally not well understood, despite the large amount of research that has been conducted (Hicks et al. 1991). Many investigators have demonstrated correlations between habitat features and salmonid abundance or production based on observational data (Lewis 1969; Stewart 1970; Nielsen 1986; Hillman et al. 1987; Newman and Waters 1989; see Fausch et al. 1988 for review). In contrast, relatively few researchers have experimentally manipulated habitat and measured changes in salmonid abundance or biomass (Reeves et al. 1991), even though modifying physical features of streams to enhance habitat for resident trout has been practiced by fishery managers in North America since at least the 1930s (Tarzwell 1937; Ehlers 1956). The best experiments conducted to date were designed to compare effects in treatment versus control sections of one stream before and after manipulation, and

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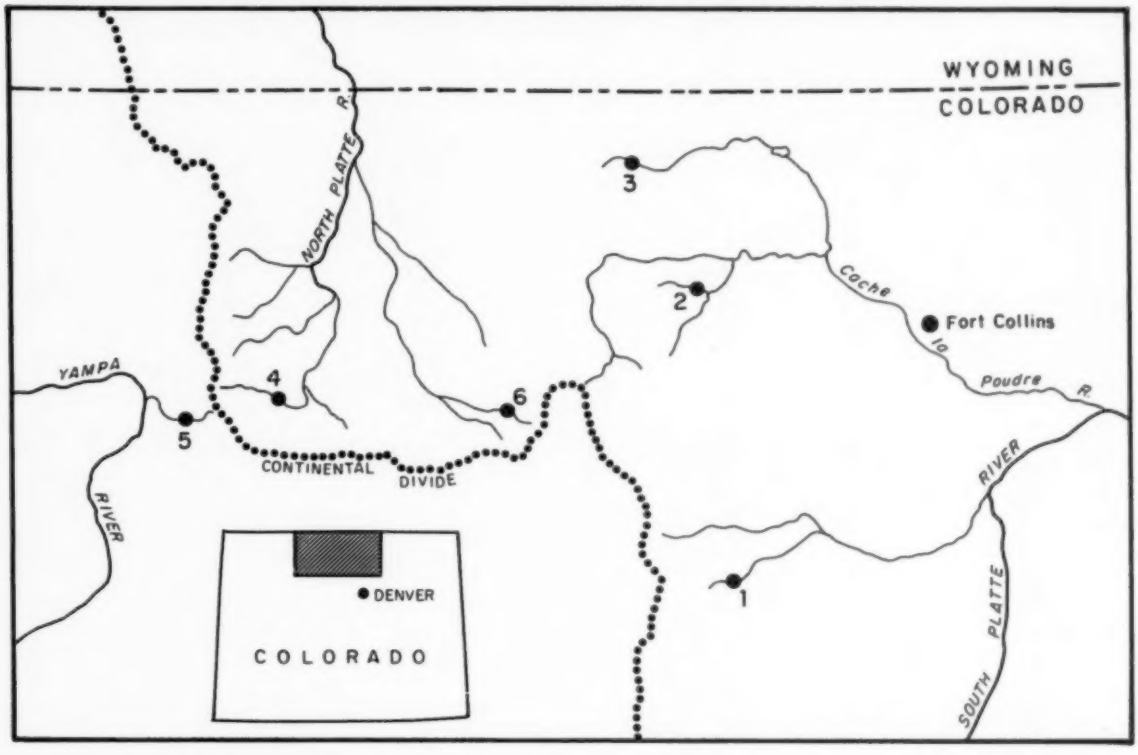
Fig. 1. Map showing the location of study streams in northern Colorado. 1, S. St. Vrain Creek; 2, Little Beaver Creek; 3, North Fork of the Cache la Poudre River; 4, Colorado Creek; 5, Walton Creek; 6, Jack Creek.



are thus replicated in time (e.g., Hunt 1971; White 1975), but none has also incorporated replication in space by simultaneously measuring these effects in many streams.

In addition to lacking replication, in only a few studies (e.g., Hunt 1971) were sufficiently detailed data collected to allow addressing the mechanisms responsible for changes in population abundance or biomass. Moreover, in a compendium of evaluations done in Wisconsin, Hunt (1988) reported that only a few researchers had also measured the response of non-salmonids to habitat enhancement, or carefully measured angler use, and emphasized the need for these data in future studies.

Log drop structures (also known as low log dams, log weirs, or low-stage check dams) are routinely installed in streams of moderate to high gradient as habitat enhancement for salmonid populations (White and Brynildson 1967). These devices are designed to scour a plunge pool below the log and impound a pool above it, and are assumed to create more and (or) better habitat for trout (Rosgen and Fittante 1986), thereby increasing their survival (Hunt 1971). Although there is some evidence that the conditions created result in increased trout abundance or biomass (Gard 1961; Burgess and Bider 1980; Maughan and Nelson 1980), there has never been an experimental evaluation of their effects.

Our purpose here is to report on an experiment, replicated in both space and time, to evaluate the effects of log drop structures on trout populations in relatively long reaches of six small, remote, high-elevation streams in northern Colorado. The experiment, begun in 1987, was designed to measure changes in abundance and biomass (i.e., the population-level response) of trout and other fish species caused by the increased habitat, relative to unmanipulated control reaches. Moreover, because wild trout populations can only increase by increased trout growth, survival, or immigration, we designed the study to test these three possible mechanisms. Finally, because angling mortality or unexpected changes in stream habitat might confound our results we also measured these characteristics in all streams.

Methods

Study sites

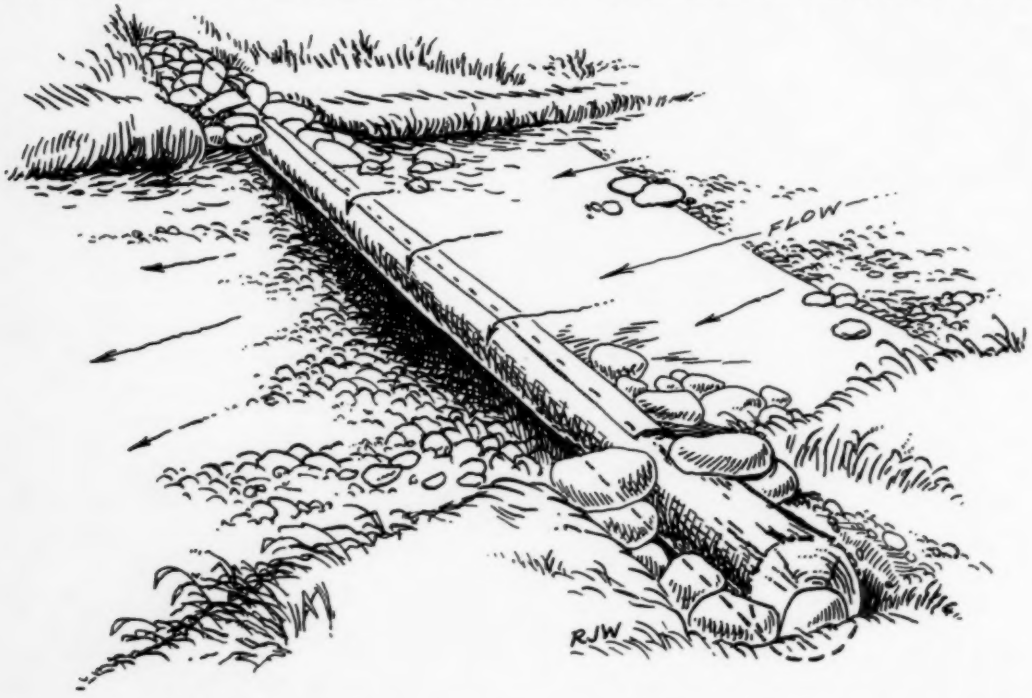
We selected study reaches on six remote, moderate-gradient (range 1–2.4%) streams in northern Colorado (Fig. 1) that had little large wood debris in the channel and few pools (Tables 1 and 2). All streams were shallow and narrow with coarse substrate and little instream cover for fish, similar to those where fishery management agencies in our region were installing log drop structures in 1985 when the study was proposed. The reaches were chosen to represent a range of elevations and habitat characteristics (e.g., cover, pool volume) to make our results broadly applicable to streams in this region. Late-summer baseflow discharge on all streams was <0.1 $m^3 \cdot s^{-1}$, and all

Table 1. Location, elevation, stream size, and composition of the salmonid community for six study streams in northern Colorado.

Stream	Longitude and latitude	Elevation (m)	Location of treatment section	Mean width (m)	Section area (m^2)		Salmonid species present
					CTL	TRT	
Beaver	40°38′N 105°32′W	2425	US	2.9	799	1097 (815)	Brk, Brn
St. Vrain	40°9′N 105°25′W	2470	US, DS	5.8	1380	2091 (2140)	Brk, Brn, Rbw
North Fork	40°49′N 105°42′W	2730	DS	3.5	818	1144 (1009)	Brk
Colorado	40°28′N 106°37′W	2775	US	2.9	772	914 (656)	Brk
Walton	40°23′N 106°42′W	2775	DS	3.7	871	903 (790)	Brk
Jack	40°25′N 105°59′W	2925	US	3.2	1064	1132 (732)	Brk, Cut

Note: Salmonid species: Brk, brook trout; Brn, brown trout; Rbw, rainbow trout; Cut, Colorado River cutthroat trout. US, upstream; DS, downstream; CTL, control; TRT, treatment. Stream codes: North Fork, North Fork of the Cache la Poudre River; Colorado, Colorado Creek; Walton, Walton Creek; Jack, Jack Creek; Beaver, Little Beaver Creek; St. Vrain, S. St. Vrain Creek. Section areas are averages over all four years (control) or three post-treatment years (treatment; pretreatment data in parentheses).

Fig. 2. Isometric drawing of a typical log drop structure as installed in six streams in northern Colorado. Arrows indicate stream flow. The end of the log in the foreground is shown without the layer of cobble and boulder used to secure it to show detail. Details of materials and construction are given in the text. Drawing by Ray J. White.



were therefore easy to wade through. All streams were relatively remote and undisturbed, although the meadows adjacent to Little Beaver Creek and Colorado Creek were occasionally grazed by cattle, and the flow regime of S. St. Vrain Creek was regulated by releases from an upstream reservoir.

The streams generally had low conductivity (range, 35–117 $\mu S \cdot cm^{-1}$ during base flow), low alkalinity (range <5–51 $mg \cdot L^{-1}$) and hardness (range 8–56 $mg \cdot L^{-1}$), and relatively neutral pH (range 7.2–8.1) when measured during base flow in August through October of 1988–1990. All streams except Jack Creek had low inorganic nitrogen (<0.02 $mg \cdot L^{-1}$ NO_2–N and NO_3–N, in most cases <0.01 $mg \cdot L^{-1}$ of both). In Jack Creek nitrate-nitrogen ranged 0.10–0.18 $mg \cdot L^{-1}$.

Naturally reproducing populations of non-native salmonids dominated the fish communities of all streams. Walton Creek, Colorado Creek, and the North Fork of the Cache la Poudre River (hereafter North Fork) contained only populations of brook trout *Salvelinus fontinalis*, while brown trout *Salmo trutta* dominated (>90%) in Little Beaver Creek (the rest were brook trout). Jack Creek supported brook trout and a small population of Colorado River cutthroat trout *Oncorhynchus clarki pleuriticus*. The fish community in S. St. Vrain Creek was composed of about equal proportions of brook trout, brown trout, rainbow trout *Oncorhynchus mykiss* (28% each), and 15% longnose sucker *Catostomus catostomus*.

Study reaches on all streams except S. St. Vrain Creek were 500 m long. These reaches were divided in half, and one of the 250-m sections was randomly designated as the treatment section (Table 1). The treatment and control sections were contiguous to ensure that pre-treatment habitat conditions were similar between the sections, except in Little Beaver Creek where approximately 200 m of unsuitable habitat separated them. South St. Vrain Creek was selected for more intensive study, so a 375-m treatment section was located between two adjacent 250-m control sections. Treatment and control sections of these lengths were judged sufficient when the study was planned because resident stream salmonids were thought to be sedentary, remaining within single pools or short stream reaches (e.g., Miller 1957; Bachman 1984).

The locations for log drop structures were planned by U.S. Forest Service hydrologists and fishery biologists, and structures were built during summer base flow in 1988 by Forest Service personnel. Ten structures were installed in the treatment sections of the North Fork, Colorado Creek, Walton Creek, and Jack Creek. Fifteen structures were built in S. St. Vrain Creek because the treatment section was 1.5 times the length on other streams. About a third of the treatment section on Little Beaver Creek had a divided channel, so 6 smaller structures were built in these channels instead of 3 (i.e., 13 structures in all) to provide about equal habitat volume compared with other streams.

Each structure consisted of a single conifer log, 25–40 cm in diameter, positioned perpendicular to the flow (Fig. 2; see Everhart and Youngs 1981; Seehorn 1985 for similar designs). A trench was excavated so that the bottom of the log rested at the deepest point of the channel cross section, and the ends of the log were embedded 1–1.5 m into each stream bank. A 2-m wide strip of 13-mm mesh hardware cloth was overlain with thick nylon cloth, stapled to the upstream face of the log, and laid upstream. Cobble and small boulders removed from directly downstream of the log were placed in a layer over the bed and banks upstream of the log to secure the structure.

Table 2. Habitat characteristics of experimental sections on six streams in northern Colorado, 1987–1990.

Stream	Section	Total cover (m)				Mean depth (m)			Mean velocity ($cm \cdot s^{-1}$)			Percent fine substrate		
		1987	1988	1989	1990	1988	1989	1990	1988	1989	1990	1988	1989	1990
North Fork	CTL	30	50	64	47	11 (9)	13 (11)	—	57 (19)	67 (20)	—	4	4	—
North Fork	TRT	30	56	105	82	15 (14)	19 (16)	—	37 (23)	57 (26)	—	3	13	—
Colorado	CTL	57	39	62	30	8 (8)	9 (9)	9 (9)	29 (15)	32 (15)	33 (16)	5	7	5
Colorado	TRT	90	109	174	89	17 (14)	27 (25)	16 (13)	14 (13)	13 (17)	18 (13)	11	37	19
Walton	CTL	—	24	33	8	12 (10)	13 (10)	13 (10)	47 (18)	45 (15)	48 (17)	2	3	2
Walton	TRT	21	116	89	67	20 (16)	21 (15)	22 (17)	25 (17)	26 (15)	30 (16)	6	8	6
Jack	CTL	31	12	29	30	8 (8)	8 (8)	10 (9)	46 (20)	58 (20)	58 (16)	0	1	1
Jack	TRT	26	26	69	67	13 (13)	11 (11)	14 (12)	29 (22)	46 (26)	48 (23)	1	0	0
Beaver	CTL	15	20	32	21	10 (8)	11 (11)	12 (9)	46 (22)	54 (18)	65 (20)	6	9	2
Beaver	TRT	41	71	112	90	13 (14)	14 (13)	16 (15)	25 (18)	34 (20)	33 (19)	23	29	17
St. Vrain	DS CTL	6	—	—	14	8 (9)	—	—	40 (17)	—	—	7	—	—
St. Vrain	TRT	13	—	—	125	14 (13)	—	—	25 (18)	—	—	9	—	—
St. Vrain	US CTL	4	—	—	5	12 (8)	—	—	42 (18)	—	—	3	—	—

Note: Stream and section codes as in Table 1. The standard deviation is shown in parentheses following mean depth and mean velocity. The percentage of points with fine substrate (sand and silt, <2 mm diameter) is shown. The 1987 data for cover in Walton Creek were collected in 1988, just before logs were installed.

All structures were installed using hand tools only to minimize disturbance to fish populations, and were spaced at intervals of about five to seven channel widths or more (except where this was prevented by large boulders in the banks) in order to mimic the spacing of pools in natural channels (Dunne and Leopold 1978; Richards 1982). All structures functioned normally and required only minor repairs during 1988 to 1990, despite high runoff from melting snow in all streams in some years. Experimental sections on all streams were posted in summer 1988 with signs spaced at 50-m intervals declaring voluntary catch-and-release, fly-and-lure-only fishing regulations.

Habitat

We used a transect method to describe the physical habitat of the streams. In 1987, width, depth, and dominant streambed material (estimated visually using a modified Wentworth classification; Orth 1983) were measured at 1-m intervals along transects perpendicular to flow starting at 0.5 m from the right bank (defined facing downstream). Transect spacings were 1 m in pools, 2 or 3 m in runs, and 3 or 5 m in riffles, depending on stream width. Lengths of each riffle, run, and pool were also measured.

In 1988, permanent transects perpendicular to flow were marked with stakes every 5 m. Depth, mean water velocity (measured at 0.6 total depth), and streambed material were measured at seven equidistant points on each transect during late summer base flow in 1988, 1989, and 1990. The depth at each bank and transect width were also recorded. In 1988, habitat was measured after the logs were constructed, as well as before construction in the treatment section of Walton Creek. Complete habitat measurements could not be made in S. St. Vrain Creek in 1988 through 1990 due to the fluctuating flow regime. Transect measurements were not made in the North Fork in 1990.

During all years, pool volumes were estimated by measuring depths at 1-m intervals on transects established within areas that were identified as pools. Transects were spaced 1–5 m apart, with wider spacing in longer pools (pools ranged up to 40 m in length). Volume was estimated by calculating the volume of cells defined around each depth measurement. Pool volumes were measured at base flow, usually the same day as transect data were collected.

We determined the amount of overhead cover for fish by measuring the lengths of five cover types capable of concealing a 15-cm fish from overhead view: undercut bank, logs, wetted brush, overhanging riparian vegetation, and boulders. We defined cover as any of these structures that were at least 15 cm wide (except undercut bank, for which 10 cm was considered adequate) and either wetted or no more than 15 cm above the water surface, with at least 15 cm of water beneath them. Riparian vegetation and brush were not measured unless they shielded at least 50% of the area beneath from overhead view.

Fish populations

We used electrofishing to obtain removal estimates of fish population size. Each section was sampled at least once each year during late summer base flow (late July to September, depending on altitude and flow). Jack and Walton creeks were not sampled in 1987, but were sampled during base flow before logs were installed in late summer 1988. Little Beaver Creek was also sampled in April or May in 1988–1990 and in July 1988. S. St. Vrain Creek was also sampled in October 1988 and May 1989.

We electrofished each section in an upstream direction using battery-powered backpack electrofishing units operating at 250–400 V DC (see Riley and Fausch (1992) for details). The ends of sections were blocked with 5-mm mesh seines before sampling to ensure population closure, and each estimate was completed in 1 day. In all cases at least three electrofishing passes were made, except for two, two-pass estimates made in the 375-m treatment section of S. St. Vrain Creek in 1987 and 1988 (the section was divided thereafter for electrofishing). A total of 10 four-pass estimates was conducted in certain sections of all streams except Colorado Creek during 1988 and 1989 (see Riley and Fausch 1992). Care was taken to ensure that all habitat was sampled and that effort remained constant on each pass. As a result, we captured the majority of the fish present and our estimates were generally precise (see Riley and Fausch 1992).

All fish captured during electrofishing were anesthetized (MS-222), measured (total length (TL) to nearest millimetre), weighed (nearest gram in 1987 and spring 1988, nearest 0.1 g thereafter), fin-clipped by section (adipose in upstream sections, left pelvic downstream; fish >69 mm only), retained in live baskets in the stream, and released near their point of capture after electrofishing was completed. In Little Beaver and S. St. Vrain creeks, all fish >125 mm TL were also marked with individually numbered Floy Fine Fabric Anchor tags after spring 1988. Trout older than age 0 that were held in live baskets overnight suffered only 0.5% mortality (n = 2122 fish, see Riley et al. 1992).

For salmonids, population estimates were calculated for two age classes (age 1 and age 2 and older) of each species, which were easily identified from length–frequency histograms. Separating age classes in this way helps avoid size-related heterogeneity in capture probability among individuals (Otis et al. 1978). For longnose suckers in S. St. Vrain Creek, age classes could not be identified, so we divided them into 'small' (<205 mm) and 'large' (≥205 mm) size classes for all estimates because there was a consistent gap in the histograms for most sampling dates.

We calculated maximum-likelihood removal estimates of trout population size for the two age classes in each section using the generalized removal estimator of Otis et al. (1978) as computed by the computer program CAPTURE (White et al. 1982). We did not compute a removal estimate if fewer than 30 fish were captured because the removal estimator performs relatively poorly at these small sample sizes (Riley and Fausch 1992). Instead, the total number of fish captured was used as the estimate in these cases.

The confidence limits of each population estimate were estimated by a procedure outlined in Burnham et al. (1987, p. 212):

$$\text{Lower 95\% confidence limit} = N/A$$

$$\text{Upper 95\% confidence limit} = NA$$

where $A = \exp(z[\ln(1 + [\mathrm{CV}(N)]^2)]^{0.5})$, N is the population estimate, $z = 1.96$, and $\mathrm{CV}(N)$ is the coefficient of variation

of N. We used the total number of fish captured as the lower confidence limit when it was greater than the limit calculated.

We estimated the biomass of each species in each section by multiplying the population estimate by the mean weight of the fish in the appropriate size class. The finite population variance of the biomass estimate (K. Burnham, Colorado State University, Ft. Collins, CO 80523, personal communication) was computed as:

$$\mathrm{VAR}(B) = B^2\left[(\mathrm{VAR}(N)/N^2) + ([(\mathrm{VAR}(w)/n)/\mathrm{AVE}(w)^2]\ (1 - n/N))\right]$$

where B is biomass (g), VAR(N) is the variance of the population estimate, VAR(w) is the variance of fish weights, AVE(w) is the mean fish weight (g), and n is the number of fish weighed. As for abundance, the total biomass of fish captured was used as the lower 95% confidence limit when greater than the limit calculated.

To test for differences in salmonid abundance and biomass in treatment relative to control sections for individual streams, we constructed a statistical contrast by subtracting pre-treatment differences in abundance between treatment and control sections from post-treatment differences. For example, in Colorado Creek, the pre-treatment (1987) difference in age-1 brook trout abundance between treatment and control sections was 61 fish, while the post-treatment difference in 1989 was 113 fish, leaving a contrast of 52. The variance of this contrast is the sum of the variances of the estimates used to compute it. We tested the significance of this contrast by computing the standard normal variate

$$z = C/(\mathrm{VAR}(C))^{0.5}$$

where C is the contrast and VAR(C) is the variance of the contrast. Many contrasts could not be calculated for certain species in S. St. Vrain Creek and for age-1 trout in other streams because fewer than 30 fish were captured.

We determined whether trout in treatment sections were in better condition than those in control sections by performing analysis of covariance on log-transformed length–weight relationships for each year that we sampled. The relationship that was fit for fish from each section was

$$\ln W = \ln a + b \ln \mathrm{TL}$$

where W is weight (g), a is the intercept, b is the slope, and TL is total length (mm).

In analysis of covariance, one first tests for a difference between slopes of the two length–weight regressions. If the slopes are not significantly different, it is then possible to test for a difference in elevation (or intercept) of the lines between the sections. A difference in slopes suggests that trout in the two sections are growing at different rates, while a significant difference in elevations indicates that fish in one section are in better condition (i.e., have a greater mean weight) at a given length than fish from the other section. We also analyzed growth of individually marked trout in S. St. Vrain and Little Beaver creeks based on recapture data.

We estimated immigration and apparent survival of trout in experimental sections on all streams from recapture rates of finclipped trout. We also calculated maximum-likelihood estimates of survival (Φ) from capture-recapture data for tagged trout in Little Beaver and S. St. Vrain creeks using the algorithms in computer program RELEASE (Burnham et al. 1987). We tested for differences in survival between sections by calculating the standard normal variate

$$z = \Phi_c - \Phi_t/[\mathrm{VAR}(\Phi_c) + \mathrm{VAR}(\Phi_t)]^{0.5}$$

We also estimated the non-centrality parameter

$$\lambda = (\Phi_c - \Phi_t)^2/(\mathrm{VAR}(\Phi_c) + \mathrm{VAR}(\Phi_t))$$

where Φ_c is the survival estimate for control section, Φ_t is the survival estimate for treatment section, VAR(Φ_c) is the variance estimate for Φ_c, and VAR(Φ_t) is the variance estimate for Φ_t. We used the Statistical Analysis System (SAS Institute 1988) to estimate the power of detecting differences in survival between treatment and control sections from the non-centrality parameter.

Angler surveys

We conducted a creel survey on four streams in 1989, and on S. St. Vrain Creek in 1990, to estimate the amount of angling that occurred. We used a stratified random sampling design, with "day of week" (weekend, weekday) as our major stratum. In 1989, the two streams that were less remote (S. St. Vrain and Little Beaver creeks) were sampled twice as often (8 d/stratum) as were the other streams (North Fork, 4 d/stratum; Jack Creek, 4 weekdays, 5 weekend days). Sampling dates were chosen randomly within a 3-month (June through August) sampling period. Angler counts were made once each 1.5 h, from 1000 to 1900 h MDT. For each of the seven counts, the clerk walked the entire study section and attempted to interview each angler counted. We used the same methods on 32 randomly chosen dates (17 weekend days, 15 weekdays) during June through August 1990 on S. St. Vrain Creek only, because few or no anglers were found on other streams in 1989.

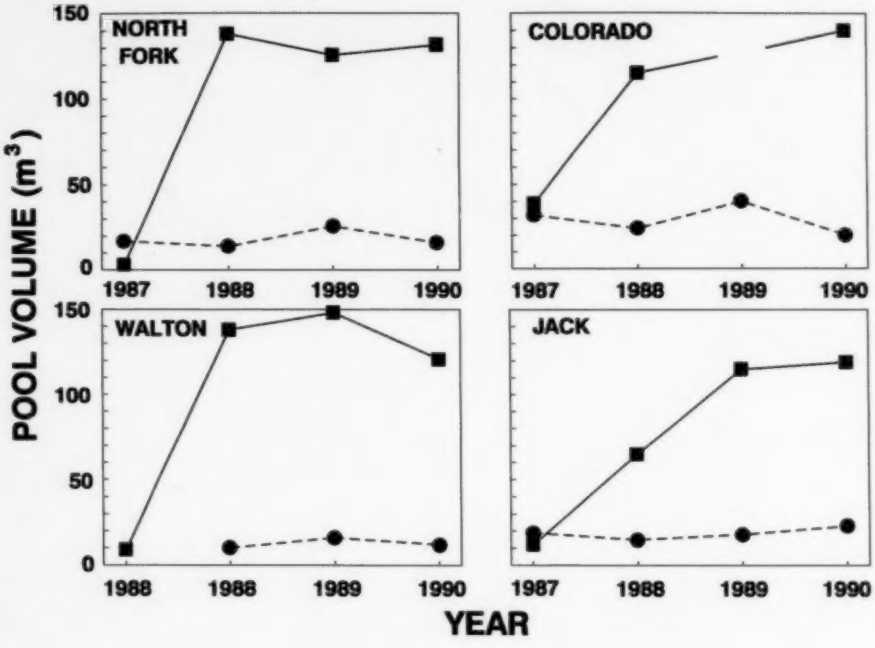
We further quantified angling with 8-mm movie cameras, set to expose one frame every 5 min, on four streams in 1989 and on all streams in 1990. In 1989, cameras were placed in the treatment sections of S. St. Vrain, Little Beaver, Colorado, and Walton creeks, and were mounted so as to photograph a long reach that included a promising pool for angling. In 1990, cameras were also installed in the treatment section of the North Fork and at a campsite in the control section of Jack Creek through which most anglers accessed the reach. Cameras were operated from June through September. Films were examined frame-by-frame using a Zeiss viewer, and only those persons judged to be anglers were counted.

Results

Habitat

Several habitat variables changed markedly in our experimental sections after log drop structures were installed in 1988. Pool volume in the treatment sections of the North Fork and Walton Creek increased more than an order of magnitude after logs were installed (Fig. 3). In 1989, beavers built dams on top of four structures in Colorado Creek, which temporarily increased pool volume to 309 m^3. The dams were removed soon after, before the annual electrofishing. Installing 10 logs caused pool volume in treatment sections of five streams to increase from less than 40 m^3 to

Fig. 3. Estimates of pool volume in 250-m treatment (solid line) and control (broken line) sections of four streams in northern Colorado. The first estimate for the treatment section of Walton Creek in 1988 and all 1987 estimates were pre-treatment. The 1989 estimate for the treatment section of Colorado Creek was not used because of the presence of beaver dams (see text).



115–150 m^3 (20 to 112–150 m^3 in Little Beaver Creek), while that in the control sections remained at 5–40 m^3 (13–22 m^3 in Little Beaver Creek). Pool volume in S. St. Vrain Creek, measured only in 1987 and 1990, increased from 8 to 238 m^3 in the treatment section, but remained at 5–30 m^3 in the control sections. Post-treatment pool volume in treatment sections was significantly greater than in control sections across streams ($P < 0.01$, by two-way ANOVA with stream and section as factors).

The amount of cover also increased significantly ($P < 0.01$, by two-way ANOVA) in the treatment sections of all streams after logs were installed, when five streams were analyzed together (Table 2). S. St. Vrain Creek was excluded because cover was measured only in 1987 and 1990, although cover increased dramatically there also. Variation in cover estimates in control sections suggests substantial year-to-year changes. While some of this variation may have been caused by measurement error and differences in baseflow discharge among years, most was likely due to actual changes in the amount of cover present. For example, the increase in cover between 1987 and 1990 in the downstream control section of S. St. Vrain Creek was the result of a log jam that trapped large woody debris.

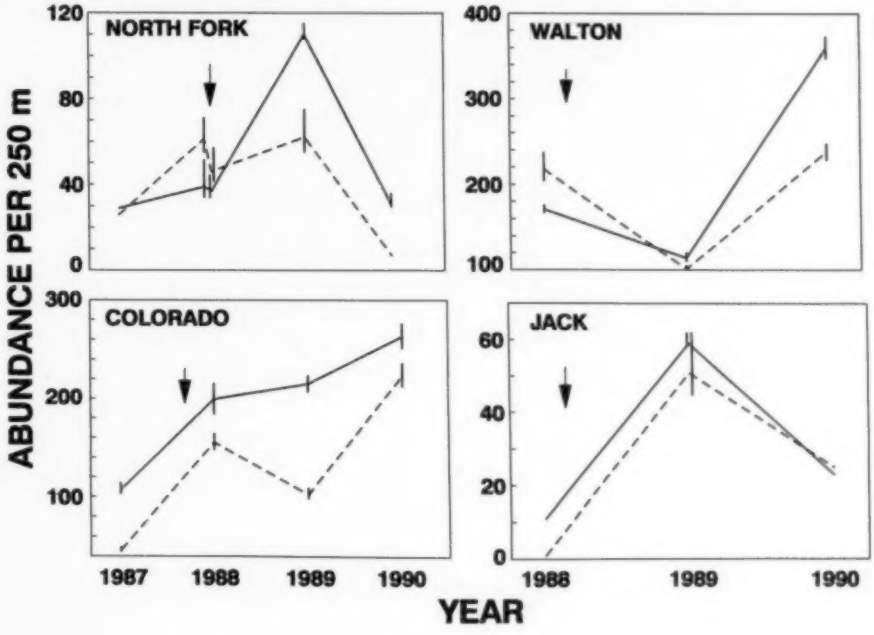
The treatment sections were also significantly deeper and had lower water velocity than control sections in the five streams after logs were installed. We made only post-treatment comparisons of depth, velocity, and substrate between sections because our sampling methods in 1987 were slightly different than in 1988–1990, although the sections on each stream were initially chosen to be similar. Mean thalweg velocity, defined as the mean of the highest velocities measured on each transect, was significantly lower in treatment than in control sections for all post-treatment comparisons ($P < 0.001$ by t-test for all, except $P < 0.05$ for the North Fork in 1989 and Jack Creek in 1989 and 1990). Mean depth was significantly greater in treatment sections in all post-treatment years ($P < 0.001$ by t-test; Table 2). The pools upstream from logs trapped bed-load sediment (silt, sand, or gravel) in all the streams. However, a higher percentage of points with fine substrate (sand or silt, <2 mm diameter) in treatment versus control sections after treatment was most evident in Little Beaver and Colorado creeks, the two with the lowest gradient, and was not evident in Jack Creek, which had the highest gradient (Table 2). The temporary increase in the treatment section of Colorado Creek in 1989 was due to the beaver dams.

Fish abundance and biomass

Abundance and biomass of adult trout (age 2 and older), and often juveniles (age 1) as well, increased significantly in the treatment sections of each of the six streams after log drop structures were installed (Figs. 4–8, see Appendix 1). Patterns of change in trout biomass were similar to abundance changes in all streams.

We used pre-treatment 1987 (1988 for Walton and Jack creeks) and post-treatment 1990 abundance and biomass

Fig. 4. Abundance estimates for age-1 brook trout in 250-m experimental sections of four high-altitude streams in northern Colorado. Solid line: treatment section; broken line: control section. Vertical lines are 95% confidence intervals. Arrows indicate when log drop structures were installed.



data from all six streams to perform a three-way ANOVA, with stream, section, and year as factors. We performed a separate ANOVA for abundance and biomass of the two age classes (all salmonids included), for a total of four analyses. In this analysis, a significant section-by-year interaction term indicates that the relationship between either abundance or biomass in the treatment and control sections was different in 1990 than before treatment, and therefore indicates a significant treatment effect. A significant effect was evident for abundance of age-2 and older trout ($P = 0.04$), but not for age-1 abundance, or biomass of either age class ($P > 0.2$). Although this indicates that, overall, only abundance of adult trout was affected by the installation of log drop structures, results from individual streams show that abundance of age-1 trout, and biomass of both age classes, also changed significantly in most cases. We first discuss results for the four streams at higher altitude (2700–2900 m) that supported primarily brook trout (Table 1), and then discuss Little Beaver and S. St. Vrain creeks, which were at lower altitude (2400–2500 m) and had other salmonid species. These groups of streams are discussed separately because of differences in the salmonid species present and physical differences in the streams due to altitude.

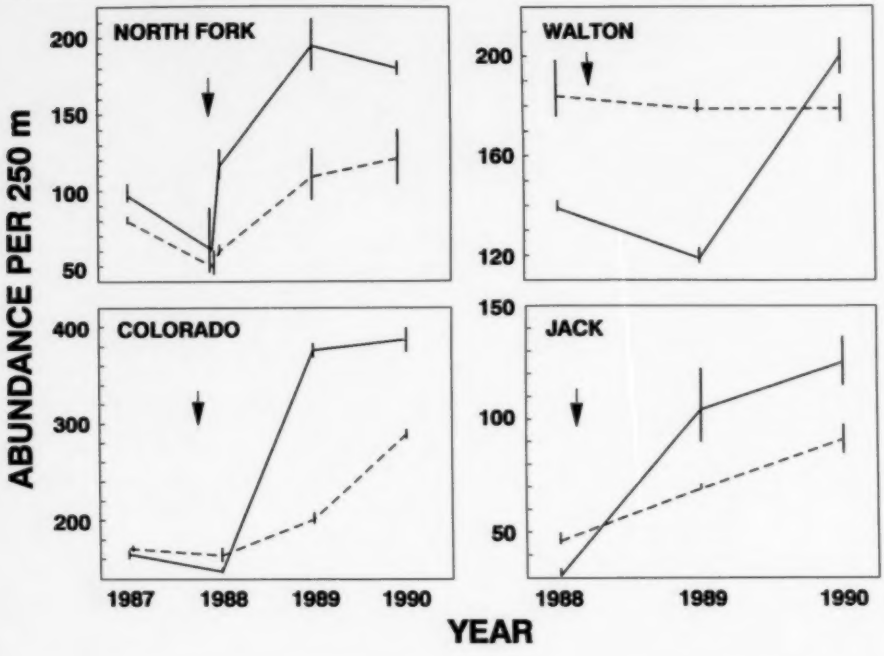
Four streams with brook trout

In the four streams that contained primarily brook trout, population responses to the experimental treatment were generally greater for adult than juvenile trout, but varied among streams (Figs. 4 and 5). Significant increases in abundance and biomass of age-1 trout in the treatment section relative to the control were observed for three of four possible contrasts (for all streams, significant contrasts had $P < 0.001$ unless otherwise designated). For age-2 and older brook trout, abundance and biomass in treatment sections increased significantly relative to controls in eight of nine contrasts ($P < 0.01$ for one contrast in North Fork). Abundance and biomass of age-2 and older brook trout were significantly greater in treatment sections than in controls in 1990 ($P < 0.05$, by two-way ANOVA), but not in 1987 ($P > 0.15$), when the four streams were analyzed together. Abundance and biomass of age-1 trout were not significantly different between treatment and control sections in either year ($P > 0.3$).

For the North Fork, statistical contrasts could not be calculated for age-1 brook trout due to low sample size in 1987, but neither abundance nor biomass appeared to increase until 1989 (Fig. 4, Appendix 1). Significant increases in abundance and biomass of age-2 and older trout, however, were found less than 1 month after structures were installed in July 1988 (Fig. 5). All post-treatment abundance and biomass differences for age-2 and older brook trout were significantly greater than pre-treatment differences ($P < 0.01$ for biomass in 1989 vs. 1987).

Abundance and biomass of age-1 brook trout in Walton Creek decreased significantly in both sections from 1988 (pre-treatment) to 1989, while abundance and biomass of age-2 and older trout remained relatively constant in both sections (Appendix 1, Figs. 4 and 5). However, in 1990 we measured large significant increases in abundance and

Fig. 5. Abundance estimates for age-2 and older brook trout in 250-m experimental sections of four high-altitude streams in northern Colorado. Solid line: treatment section; broken line: control section. Vertical lines are 95% confidence intervals. Arrows indicate when log drop structures were installed.



biomass of both age classes in the treatment section. Differences in abundance and biomass of both age classes between the treatment and control sections of Walton Creek were significantly greater in 1990 than in 1988, while 1989 differences were greater for age-1 brook trout only.

In Colorado Creek, abundance and biomass of age-1 brook trout increased in both sections each year after 1987, except for a significant decrease in the control section in 1989 (Appendix 1, Fig. 4). Dramatic increases in abundance and biomass of age-2 and older brook trout in the treatment section occurred during the year after logs were installed (1988–1989; Appendix 1, Fig. 5). In 1989, the treatment section of Colorado Creek was sampled immediately after removing four beaver dams that had impounded large volumes of water, which may have affected the annual estimates in this section. Statistical contrasts showed that abundance and biomass of age-2 and older brook trout in the treatment section increased significantly, relative to the control, in 1989 and 1990 versus 1987, while age-1 abundance did so only in 1989.

In Jack Creek, abundance of age-1 brook trout varied widely among years but remained similar in the two sections (Fig. 4). Too few fish were caught in either section in 1988 (pre-treatment) to calculate contrasts. Abundance and biomass of age-2 and older brook trout in the treatment section were significantly greater in 1989 and 1990 than in 1988 (Appendix 1, Fig. 5). Differences in age-2 and older brook trout abundance and biomass between the treatment and control sections were significantly greater in both 1989 and 1990 than in 1987. In 1988 and 1990, too few (<30) cutthroat trout were captured in Jack Creek to calculate population estimates, so results are not shown here.

Little Beaver and S. St. Vrain creeks

We sampled Little Beaver and S. St. Vrain creeks during spring before snowmelt runoff in two or three years, but moderate flows and cold water made electrofishing difficult and reduced capture probabilities. On S. St. Vrain Creek, equipment failures resulted in poor estimates for the treatment section in summer 1988, while cold water and ice compromised the results of all estimates in fall 1988. We therefore present only late summer estimates for both streams, and exclude the 1988 estimates for S. St. Vrain Creek. We also include the early July 1988 estimate for Little Beaver Creek.

Abundance of age-1 brown trout in both sections of Little Beaver Creek increased in late summer 1988, 1989, and 1990 relative to 1987 (Fig. 6). Statistical contrasts could not be calculated for age-1 trout because too few fish were caught in 1987 to calculate variances. Abundance of age-2 and older brown trout was similar in the two sections until 1989, when significant increases occurred in the treatment section. Statistical contrasts showed that abundance and biomass of age-2 and older brown trout in the treatment section increased significantly, relative to the control, in 1989 and 1990.

Changes in salmonid abundance in S. St. Vrain Creek varied by species. The large number of abundance estimates

without variances prevented us from calculating contrasts in many cases. Abundance of age-1 brook trout and brown trout increased significantly in the treatment section between 1987 and 1990 but remained similar or decreased in the controls, except for age-1 brown trout in the downstream control section, which also increased (Fig. 7). Contrasts indicated that the abundance and biomass of age-1 brook and brown trout in the treatment section were significantly greater, relative to the upstream control, in 1990 compared with 1987 ($P < 0.01$ for both). Abundance and biomass of age-1 brown trout did not increase significantly from 1987 to 1990 in the treatment section relative to the downstream control section ($P = 0.79$ for abundance, 0.47 for biomass). Abundance of age-1 rainbow trout was always too low to calculate population estimates, although it appeared to decrease in all sections after 1987.

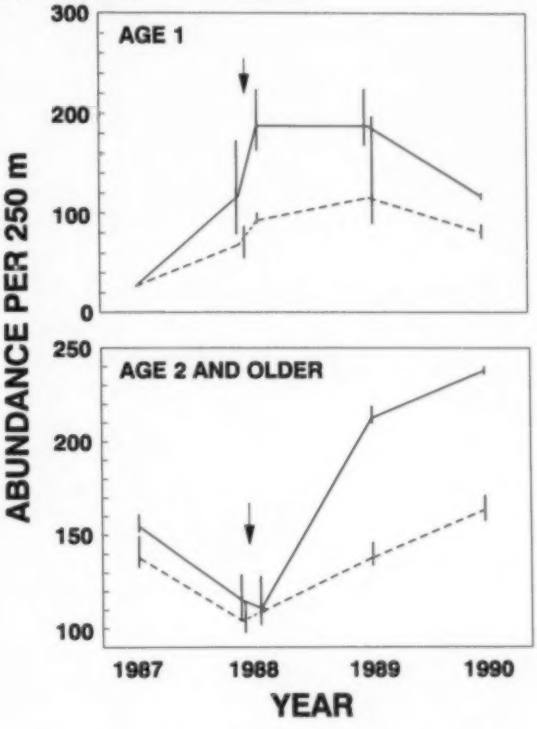
Abundance of age-2 and older brook and brown trout in the treatment section of S. St. Vrain Creek increased significantly between 1987 and 1990, with brook trout having the greater response (Fig. 8). Abundance of age-2 and older trout of all species remained similar in control sections during this period. Contrasts indicated that age-2 and older brown trout abundance and biomass were significantly greater, relative to controls, in 1989 and 1990 compared with 1987. Abundance and biomass of age-2 and older rainbow trout were significantly greater in the treatment versus upstream control section only in 1990 ($P < 0.05$ for abundance, $P < 0.01$ for biomass, $P > 0.35$ for both in 1989 vs. 1987). No such contrast could be calculated for the downstream control section due to low abundance.

Abundance of small (<205 mm) longnose suckers remained similar in the control sections of S. St. Vrain Creek throughout the experiment, while increasing dramatically (>200%) in the treatment section in 1989 and 1990 versus 1987 (Fig. 7). The significance of these changes cannot be assessed because in all cases too few small suckers were captured to calculate estimates and confidence intervals. Abundance of large (≥205 mm) suckers declined markedly in all sections (Fig. 8), but contrasts were not possible because too few were captured in 1989 and 1990.

Abundance of large trout

To put our results in perspective for managers, we compared the number of large trout that might be of interest to anglers (>200 mm in S. St. Vrain and Little Beaver creeks, >150 mm in the four brook trout streams because of a smaller terminal length in these populations) that were captured in treatment versus control sections. Most age-2 and older trout were captured in three electrofishing passes (e.g., 96% on average for 14 estimates reported in Riley and Fausch 1992) so we assumed for this analysis that nearly all large trout were captured. The installation of logs increased the abundance of large trout, relative to pre-treatment abundance, in the treatment sections more than control sections of the North Fork (77% treatment, 13% control), Walton Creek (49% treatment, 20% control), and Jack Creek (231% treatment (1989–1990 average), 36% control; see Riley 1992 for data). Numbers of large trout declined in both sections of Colorado Creek, and no changes occurred in maximum length of brook trout in any of the streams (range 195–238 mm in all streams).

Fig. 6. Abundance estimates for age-1 (top panel) and age-2 and older (bottom panel) brown trout from 250-m experimental sections of Little Beaver Creek. Solid line: treatment section; broken line: control section. Vertical lines are 95% confidence intervals. Arrows indicate when log drop structures were installed.

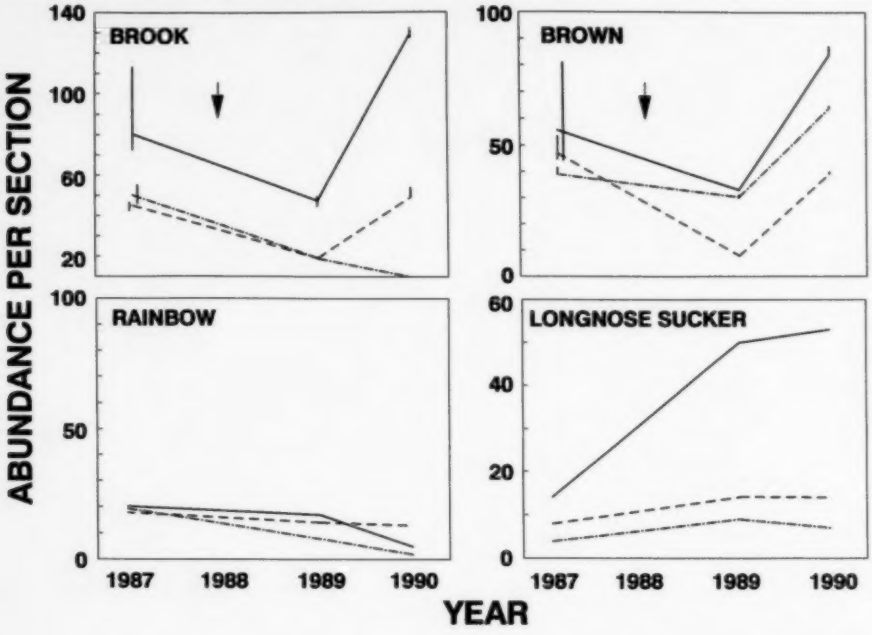


The number of large brown and rainbow trout in the treatment section of S. St. Vrain Creek increased (by 43 and 31%, respectively) after log drop structures were installed, whereas their abundance in the control sections remained similar or decreased. The maximum length of brook trout in the treatment section also increased from <211 mm in 1987 and 1988 to 230–265 mm in 1989 and 1990, while remaining similar through these years in the control sections (200–221 mm). The maximum lengths of brown trout (241–281 mm) and rainbow trout (234–281 mm) changed little among sections or through time. The number of large brown trout in both sections of Little Beaver Creek increased slightly after 1987, but more so in the control than in the treatment section, and maximum size of brown trout remained similar in both treatment (263–288 mm) and control (323–335) sections.

Trout growth

Based on analysis of covariance of log-transformed length–weight relationships, there is little evidence to suggest that installing log drop structures resulted in better growth of trout in the four high-altitude streams with brook trout. For example, pre-treatment (1987) data suggest that trout in the treatment section of the North Fork had a significantly greater mean weight at a given length than

Fig. 7. Abundance estimates for age-1 trout of three species and small (<205 mm total length) longnose suckers in the 375-m treatment section and 250-m control sections of S. St. Vrain Creek. Solid line: treatment section; dashed line: upstream control section; dot-dashed line: downstream control section. Vertical lines are 95% confidence intervals. Arrows in top panels indicate when log drop structures were installed.



in the control because slopes were similar ($P = 0.88$), but the treatment intercept was significantly ($P = 0.04$) greater. However, by July 1988 (also pre-treatment) relationships for the two sections were not significantly different ($P > 0.4$ for both). Data from the first two post-treatment samples from this stream indicate that trout in the treatment section were growing faster than in the control (i.e., slopes were significantly greater, $P < 0.03$) but in both cases the intercepts were less in the treatment than control, so the lines cross. By 1990, the relationships were not significantly different in either slope or intercept ($P > 0.70$). Although these results indicate that trout in the North Fork treatment section were gaining weight significantly faster than those in the control in 1988 and 1989, the differences in weight predicted for a 150-mm trout are <2 g in all cases. Length–weight regression lines for the other three streams often crossed as well (Riley 1992), and differences in predicted weights were also <2 g in all cases, indicating that any differences were biologically insignificant.

We also tested for differences in trout growth between the treatment and control sections of Little Beaver Creek, by comparing the weight gained by tagged brown trout (>125 mm TL) during two periods, September 1988 to September 1990 (treatment, $n = 19$; control, $n = 17$), and September 1989 to September 1990 (treatment, $n = 84$; control, $n = 47$). In all cases, weight gain was not significantly related to length at first capture ($r^2 < 0.1$, $P >> 0.05$, by linear regression), so simple means for each section were compared for each time period. From 1988 to 1990, the mean weight gain for trout in the control section (mean = 37.2, SD = 24.8) was higher than in the treatment section (mean = 30.6, SD = 17.5). The same pattern was evident from 1989 to 1990 (control: mean = 22.7, SD = 9.2; treatment: mean = 18.0, SD = 9.3), indicating that no growth benefit associated with log drop structures was detectable for tagged brown trout in Little Beaver Creek.

We performed similar analyses for S. St. Vrain Creek, but only for the September 1988 to September 1989 period because sample sizes were too small for other comparisons. Weight gain by tagged brook trout was not significantly related to initial length or weight in the two control sections ($P > 0.65$, by linear regression), but was so at $P < 0.15$ for both in the treatment section (probability of type I error was increased to improve statistical power to detect slope different from zero). Both initial weight ($P = 0.01$, by ANOVA) and weight gain ($P < 0.01$) of tagged brook trout in the treatment section were significantly greater than in the control sections ($P < 0.05$, by GT2 test), except that weight gain was not significantly different in the treatment and upstream control sections ($P > 0.05$). However, because weight gain was positively related to initial length and weight in the treatment section, the significant difference in weight gain may be at least partially due to the greater initial weight of brook trout in the treatment section. Weight gain of brown and rainbow trout was significantly related to both initial length and initial weight in all sections (i.e., larger trout gained weight faster, $P < 0.05$, by linear regression, for all except $P < 0.15$ for rainbow trout in upstream

Fig. 8. Abundance estimates for age-2 and older trout of three species and large (≥205 mm total length) longnose suckers in the 375-m treatment section and 250-m control sections of S. St. Vrain Creek. Solid line: treatment section; dashed line: upstream control section; dot-dashed line: downstream control section. Vertical lines are 95% confidence intervals. Arrows in top panels indicate when log drop structures were installed.

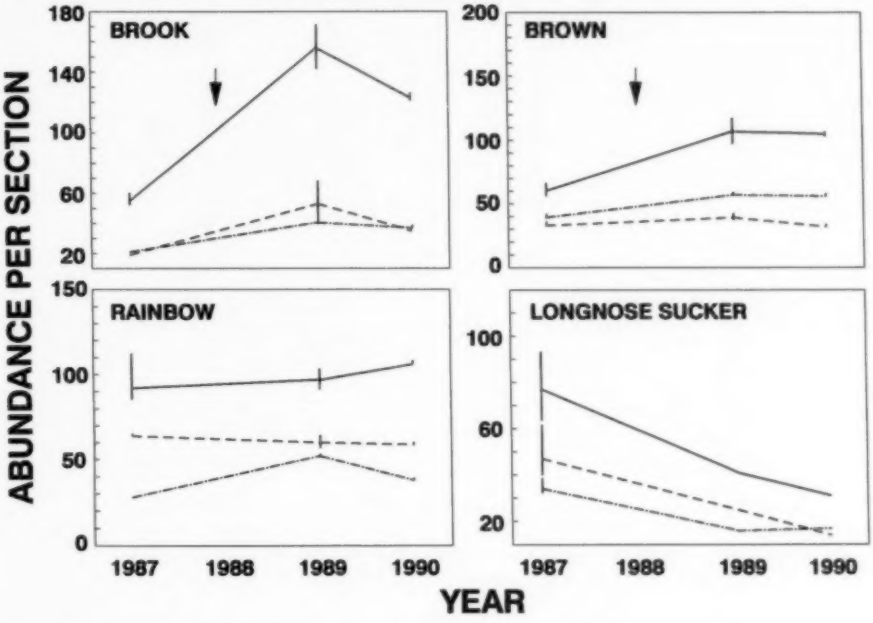


Table 3. Number and percentage (in parentheses) of fin-clipped age-2 and older brook trout that were recaptured in experimental sections of four streams in northern Colorado during annual sampling in July–August each year.

	1988		1989		1990	
Stream	TRT	CTL	TRT	CTL	TRT	CTL
North Fork	34 (42.5)	36 (36.4)	56 (40.6)	29 (31.2)	66 (24.0)	34 (26.0)
Colorado	65 (24.2)	62 (28.8)	114 (36.2)	73 (30.2)	157 (30.5)	91 (31.8)
Walton	—	—	62 (22.1)	139 (36.6)	77 (35.0)	112 (41.6)
Jack	—	—	9 (29.0)	13 (23.2)	39 (30.2)	38 (30.2)

Note: Stream and section codes are as in Table 1. Data are from Riley et al. (1992).

control), except for brown trout in the upstream control ($P > 0.60$), making an ANOVA analysis among the sections impossible. However, neither mean initial weight nor mean weight gain of brown or rainbow trout was significantly different among the three sections ($P > 0.45$, by ANOVA). In summary, there is little evidence that installing log-drop structures increased trout growth in any of the streams.

Estimates of survival

In three of the four streams with primarily brook trout, numbers of resident trout increased in treatment sections relative to controls after logs were installed (Table 3). Because we captured and fin-clipped most age-1 and older trout present in the sections each year (see Riley et al. 1992), the percentage remaining (age-2 and older) of the total number of marked trout known to have been present on the previous sampling occasion provides an estimate of apparent survival (i.e., survival plus net immigration). Numbers of resident trout could not be used to estimate survival in Little Beaver and S. St. Vrain creeks because fish were also fin-clipped during spring population estimates, when captured probabilities were low.

We recaptured a larger percentage of previously marked trout in the treatment section than in the control section of the North Fork, Colorado Creek, and Jack Creek in 1989, which suggests that survival may have been better where log drop structures were installed. A smaller percentage of trout, however, were recaptured in the treatment section of Walton Creek than in the control in 1989 and 1990. The 1990 estimates for the other three streams were similar for

Table 4. Maximum-likelihood estimates of survival for tagged trout from Little Beaver and South St. Vrain creeks, Colorado.

Stream and species	Period	Survival		*P*	Power
		Treatment	Control		
Beaver Brn	07/88–09/88	0.73 (0.080)	0.55 (0.056)	>0.05	0.46
	09/88–04/89	0.65 (0.067)	0.87 (0.093)	<0.05	0.50
	04/89–09/89	0.59 (0.063)	0.33 (0.052)	<0.05	0.89
	09/89–09/90	0.82 (0.057)	0.79 (0.063)	>0.05	0.06
St. Vrain Brk	07/88–10/88	0.60 (0.073)	0.65 (0.064)	>0.05	0.09
	10/88–05/89	0.83 (0.067)	0.74 (0.062)	>0.05	0.16
	05/89–09/89	0.57 (0.061)	0.56 (0.070)	>0.05	0.05
St. Vrain Brn	07/88–10/88	0.65 (0.079)	0.51 (0.048)	>0.05	0.31
	10/88–05/89	0.81 (0.102)	0.78 (0.054)	>0.05	0.06
	05/89–09/89	0.46 (0.073)	0.49 (0.053)	>0.05	0.06
St. Vrain Rbw	07/88–10/88	0.53 (0.077)	0.54 (0.055)	>0.05	0.05
	10/88–05/89	0.83 (0.079)	0.89 (0.081)	>0.05	0.09
	05/89–09/89	0.56 (0.065)	0.53 (0.076)	>0.05	0.06

Note: Estimates for SSV control sections are mean survival estimates for the two sections. Numbers in parentheses are standard errors. The significance of differences in survival between treatment and control section (*P*), and the power of these tests to detect differences in survival are also shown. Species and stream codes are as in Table 1.

Table 5. Percentage of age-2 and older trout captured during 1988 through 1990 that bore no fin clip from previous sampling in six streams in northern Colorado.

Stream	Species	1988		1989		1990	
		TRT (%)	CTL (%)	TRT (%)	CTL (%)	TRT (%)	CTL (%)
North Fork	Brk	72	40	65	52	51	52
Colorado	Brk	48	56	54	61	42	58
Walton	Brk	—	—	39	18	55	25
Jack	Brk	—	—	84	81	48	52
Beaver	Brn	61	46	52	48	40	42
St. Vrain	Brk	59	53,52	30	23,49	60	38,57
St. Vrain	Brn	51	46,36	46	54,49	45	43,46
St. Vrain	Rbw	33	36,35	25	22,26	9	13,35

Note: Data for the four streams with primarily brook trout are after Riley et al. (1992). For S. St. Vrain Creek, the downstream control section is listed first, followed by the upstream control. Stream and section codes are as in Table 1.

treatment and control sections, suggesting that any survival benefit associated with these structures may have occurred only for one year, or may vary among years.

Maximum likelihood estimates of survival for tagged trout from the Little Beaver and S. St. Vrain creeks suggest that there was little survival benefit associated with log drop structures. The difference in trout survival in treatment versus control sections of St. St. Vrain Creek was <9% in eight of nine cases, and always <15% (Table 4). In Little Beaver Creek, brown trout survival was significantly greater in the control than in the treatment section between September 1988 and April 1989, while survival was significantly greater in the treatment section between April and September 1989. For all but 1 of 10 cases where the difference in survival between sections was <15%, the probability of detecting a difference of the magnitude tested at $P = 0.05$ (i.e., power) was <0.16. However, the power to detect survival differences ≥18% in the other three cases was relatively high (0.46–0.89).

Trout movement

Because we captured most age-1 and older trout present in our sections each time we electrofished (see Riley and Fausch 1992; Riley et al. 1992), most age-2 and older trout that did not bear fin clips from previous sampling occasions must have been immigrants. The percentage of brook and brown trout captured during 1988 through 1990 that bore no fin clip ranged from 17 to 84%, with a median of 52% (Table 5). Percentages of immigrant rainbow trout in S. St. Vrain Creek were lower (range 9–36%, median 26%). In 7 of 10 cases where differences in the percentage of immigrant trout between treatment and control sections were >10%, treatment sections contained the higher percentage of immigrants (North Fork 1988 and 1989, Walton

1989 and 1990, Little Beaver 1988, S. St. Vrain brown trout 1988, S. St. Vrain brook trout 1990).

Some trout moved from control to treatment sections or vice versa, but the percentage that bore fin clips indicating that they had moved since initial capture was consistently low (median = 6.6%, range = 0–18.3%) in the four streams with brook trout (see Table 2 of Riley et al. 1992). Moreover, in 4 of 10 comparisons the percentage of trout bearing fin clips from the other or both sections was higher in the control than treatment section, providing little evidence to suggest that the treatment section was consistently attracting large numbers of trout from the adjacent reaches.

Angler surveys

South St. Vrain Creek supported the most angling, but our results indicated that angler use was less than one angler per day in both 1989 and 1990. Most anglers were observed on weekends; we counted anglers on only 2 of 9 weekdays in 1989 (one was a holiday), and on 2 of 15 in 1990. The low and sporadic angling precluded meaningful variance estimates for any measures of angling for this and all other streams. Four fish were observed killed in 17 d of the 1989 survey (0.24/d), all in the treatment section, and seven fish were observed killed in 32 d in 1990 (four in treatment, 0.13/d; three in control, 0.09/d). More anglers fished in the treatment sections than the control sections (10 vs. 2 in 1989; 25 vs. 15 in 1990), and spent more time there (17.5 vs. 2.5 h in 1989; 36.1 vs. 23.0 h in 1990). These differences in angling effort could make the observed abundance increases conservative (cf. Figs. 7 and 8). We suspect that the effects of angling mortality were small, however, when compared with natural mortality.

Creel surveys on Little Beaver Creek, Jack Creek, and the North Fork in 1989 suggested that angling pressure was very low. Four anglers (0.4/day) were observed in each section on Jack Creek, and fished more in the treatment section than in the control (5 vs. 1 h). No anglers were observed on either the North Fork or little Beaver Creek, and no fish were observed killed on any of these streams. We very rarely encountered anyone when we visited our study sections (except for S. St. Vrain Creek) for electrofishing and habitat measurements each year, which further suggests that few anglers visited them.

The 8-mm motion-picture cameras we used often malfunctioned, but the quality of the films that were exposed was good because electrofishing crews were clearly visible. The films, like the creel survey data, indicated that angler use was very low. No anglers were ever observed during 1989–1990 on films from the North Fork (82 d of filming), Colorado Creek (99 d), or Walton Creek (143 d). Only four anglers were observed on a total of 2 of 62 d of filming in Little Beaver Creek, and four anglers were observed on 2 of 75 d on Jack Creek (<0.1 anglers per day on both streams). The highest angling use was on St. St. Vrain Creek (five anglers on 4 of 19 d; 0.26 anglers/day), although relatively few days of film were properly exposed there.

Discussion

Lack of experimental evaluation is a problem common to most fishery management techniques, largely because researchers must measure fish populations over large spatial and temporal scales to detect their full response. The most complete evaluations of stream habitat enhancement have been done in individual midwestern trout streams (e.g., Shetter et al. 1946; Hale 1969; Hunt 1971; White 1975), and indicate that populations may not respond fully for 5–7 years after treatment (Hunt 1976). Therefore, even though we measured large and significant increases in adult trout abundance within 2 years after log drop structures were installed in all streams, we may have not yet recorded the full population response.

Only a few investigators have attempted to quantify the habitat changes associated with log drop structures. Gard (1961) found reduced velocities and increased depth in impounded sections, as we report here, and also found that logs increased the amount of silt. Lere (1982) reported that mean velocities were significantly lower and mean depths were significantly higher in a section of a Montana stream with log drop structures than in a nearby control reach. He also found that the amount of cover in the treated section was 63% greater than that of the control section, although this difference was not significant. Several habitat changes that we observed, such as increased depth, pool volume, and cover (Table 2), corroborate these findings. These variables have also been identified by correlative habitat models as important features that are related to high trout abundance or biomass (Hunt 1971; Fausch et al. 1988).

Fish population increases

Our results show that installing log drop structures significantly increased the abundance of adult trout in treatment sections when all six streams are considered together, and also increased the abundance and biomass of adult brook trout in the four high-altitude streams. We also observed significant increases in abundance and biomass of either one or both age classes of brook trout in treatment sections relative to control sections when each of the four high-altitude streams was considered individually (Figs. 4 and 5). The exact nature of the population response and the relative effects on the two age classes, however, varied among streams, a finding that might have been obscured if we had divided these populations into biologically less meaningful size classes (e.g., >200 mm). The increase in abundance of age-2 and older brook trout after installing log drop structures was consistent among streams, while the effects on age-1 brook trout were more variable. This was perhaps due to effects of flow or other factors on recruitment, such as Nehring and Anderson (1993) found for brown and rainbow trout in 11 rivers throughout Colorado.

Adult brown trout abundance and biomass also increased significantly after treatment in Little Beaver Creek (Fig. 6). As in the four high-altitude streams, the changes in age-1 brown trout were more variable, although they could not be tested statistically due to low sample sizes. Statistical contrasts also could not often be calculated for S. St. Vrain Creek, but the results indicate that brook trout and age-2 and older brown trout increased the most after log installation, while rainbow trout of both age classes either benefitted less or decreased in abundance (Figs. 7 and 8). Lere (1982) obtained similar results in a Montana stream; while total salmonid abundance and biomass were significantly greater

in a section with log drop structures as compared with a control, rainbow trout abundance and biomass were not. Differences in the responses of the three trout species in S. St. Vrain Creek might be due to several factors, including differences in habitat selection or differential angling susceptibility and mortality. For example, rainbow trout select microhabitats with swifter current velocity than do brook trout (Cunjak and Green 1983) or brown trout, which may explain their lesser response to added pool habitat that inundated runs and riffles. While we cannot fully evaluate these hypotheses, our results indicate that the three species of trout in S. St. Vrain Creek responded differently to the log drop structures.

The number of large longnose suckers decreased in all sections of S. St. Vrain Creek between 1987 and 1990. We suspect that this was due to their susceptibility to injury by electrofishing and not an effect of our habitat manipulation. Many adult suckers captured after 1987 had spinal deformities, a phenomenon rarely observed in salmonids on this stream. In contrast, abundance of small longnose suckers increased in the treatment section but remained similar in the two control sections, probably as a result of the deep water and decreased water velocities caused by the log drop structures.

Because log drop structures are installed by managers to increase biomass of trout for anglers, their efficacy is often assessed by examining effects on the abundance of fish larger than a certain size, say 200 mm. Evaluations of other stream habitat improvement methods such as log covers and deflectors have shown marked increases in the number of catchable trout in treated sections of Wisconsin streams (Hunt 1988). We also found that the number of brook trout >150 mm increased more in the treatment sections (49–231% increases) of three of the four high-altitude streams as compared with adjacent control sections (13–36% increases). The number of brown and rainbow trout >200 mm in the treatment section of one lower-altitude stream, S. St. Vrain Creek, also increased substantially (31–43%) while numbers in control sections changed little. We suspect that fishery managers would consider the increases significant. Moreover, it is possible that the full population response has not yet occurred, as trout in similar Colorado mountain streams grow slowly and live to be more than five years old (Scarnecchia 1983).

Mechanisms accounting for population increases

Growth

A primary objective of this study was to evaluate the mechanisms that caused the changes observed in trout populations after log drop structures were installed. One such mechanism might be better growth of resident trout leading to greater biomass in treatment sections. Only brook trout in S. St. Vrain Creek grew faster in treatment than in control sections, although evidence for this was equivocal. Comparisons for the other species in S. St. Vrain Creek and for brown trout in Little Beaver Creek, all based on tag recaptures, clearly indicated that trout in the treatment sections grew no faster than those in the controls. The differences in length–weight relationships of brook trout from treatment and control sections of the four high-altitude streams were so small as to be biologically insignificant, and also may have been affected by the high rate of trout movement (see below). Overall, our results indicate that trout growth and condition were generally unaffected, at least for 2 years after the habitat manipulation. The lack of an effect of habitat enhancement on trout growth or condition was also reported by Saunders and Smith (1962), Hunt (1969), Burgess and Bider (1980), and Lere (1982), although Gard (1961) found that trout introduced into a reach of Sagehen Creek where log dams were added had higher condition factors than those from a nearby unmanipulated reach. However, trout were stocked into the treated reach at less than half the density of the control reach, so the comparison was not entirely valid.

Survival

Pools created by log drop structures are thought to increase overwinter survival of salmonids, a second plausible mechanism to explain the increase in trout populations. Because many salmonid species have been observed to occupy deeper, lower velocity habitats at low temperatures (Campbell and Neuner 1985; Johnson and Kucera 1985; Cunjak and Power 1986; Baltz et al. 1987, 1991; Hillman et al. 1987; Heggenes and Saltveit 1990; Nickelson et al. 1992*a*), this seems to be a reasonable hypothesis, although Nickelson et al. (1992*b*) suggest that plunge pools do not create suitable winter habitat conditions for juvenile coho salmon in Oregon streams. Saunders and Smith (1962) found an increase in the number of brook trout (primarily ages 0 and 2 and older) that remained in a section of a Prince Edward Island stream after log dams were installed.

If installing log drop structures results in better survival, one would expect the number of resident trout to increase in treatment sections relative to controls, which occurred in the North Fork and Colorado and Walton creeks (Table 3). One would also expect a higher recapture rate of marked fish in treatment than in control sections. Percentages of trout recaptured were slightly higher (6–9%) in the treatment sections of the North Fork and Colorado and Jack creeks from 1988 to 1989. However, in 1990 the percentages of brook trout marked the previous year that were recaptured were similar in treatment and control sections of these streams, which suggests that the relative survival in the two sections had stabilized. We emphasize, however, that these data do not allow us to separate mortality from emigration.

In S. St. Vrain and Little Beaver creeks we were able to calculate maximum-likelihood survival estimates based on mark-recapture data for tagged trout. We have no reason to suspect that Floy tag loss, which we estimate to be 10–15% per year (S.C. Riley and K.D. Fausch, unpublished data), varied among sections, and it would therefore not affect the validity of our tests. In only one case (Little Beaver Creek spring to fall 1989; Table 4) was survival higher in the treatment section, and this was not during winter. In fact, overwinter survival of brown trout was significantly higher in the control section of Little Beaver Creek during winter 1988–1989. Moreover, in 9 of the other 11 comparisons for these two streams, survival in treatment and control sections differed by <10% (Table 4). These results suggest that increased overwinter survival is an unlikely

mechanism to explain the increases in abundance that we have measured thus far.

Movement

A third possible mechanism that could explain the increases in trout populations is immigration. Our results indicate that, on average, approximately half of the adult brook trout and brown trout present in experimental sections had immigrated since the previous year (Table 5). In contrast, a median of 6.6% (see above) had moved from control to treatment sections or vice versa. The deeper, lower velocity habitat with more cover that was created by the log-drop structures apparently induced trout moving from relatively long distances to settle in these sections, rather than simply attracting large numbers of trout from adjacent 250-m reaches. Although increased survival may have played a role in the four high-altitude streams (Table 3), our data suggest that the primary short-term benefit of log drop structures was to create habitat attractive to immigrant trout. Moreover, the high rates of immigration are a more likely explanation than increased survival for the rapid increases in trout abundance found in all streams except Walton Creek (Figs. 4–8).

Other investigators have also suggested that movement plays an important role in trout population response to habitat enhancement. Hunt (1965) found high rates of dispersal of age-0 brook trout among four long (>1 km) sections of a Wisconsin stream, and indicated that adult trout moved upstream to a manipulated section to find suitable spawning gravel (Hunt 1974). Hunt (1969, 1974; see also Table 2 in Chapman and Bjornn 1969) also reported that emigration of juvenile and adult brook trout from a manipulated section declined after habitat enhancement, even as abundance increased. Although he attributed most of the increased abundance to higher overwinter survival, the possibility that movement was also important cannot be ruled out without direct measurements of survival and immigration. Burgess (1985) and Burgess and Bider (1980) found high rates of brook trout net upstream immigration into matched 100-m sections of a divided Quebec stream, but much lower rates of net upstream emigration from the section where pools were created and overhead cover added. Both of these studies suggest that movement may be an important mechanism by which trout populations respond to enhanced habitat. However, more detailed research will be needed to separate survival from immigration in future studies.

The high movement rates that we observed have important consequences for the scale at which stream enhancement devices should be evaluated. While we observed localized increases in density after log drop structures were installed, this does not necessarily indicate improved habitat quality (Van Horne 1983; Hobbs and Hanley 1990). If trout are indeed mobile, the effects of the manipulation on an entire trout population will be difficult to predict based on research in short stream reaches (cf. Hunt 1974). Although our study sections were relatively long (500 m), the high movement rates suggest that the effects of stream habitat enhancement must be monitored at even larger scales.

Population increases in control sections

In addition to increases in trout populations in treatment sections, we also found significant increases from pretreatment levels of abundance and biomass in at least one age class of trout in the control sections of all streams except Walton Creek (Appendix 1; Figs. 4–8). These increases might be the result of some combination of three factors: (1) catch-and-release fishing regulations, (2) effects of our treatment, or (3) normal population fluctuations. The importance of the catch-and-release fishing regulations is probably small, because angling pressure was low on all streams except S. St. Vrain Creek. Moreover, age-1 trout were too small to be of interest to anglers on any stream (ca. <150 mm in S. St. Vrain Creek, <125 mm in all others).

It is possible that the log drop structures are somehow affecting the control section, perhaps by serving as a winter refuge for trout that subsequently emigrate to the control section for the summer. However, our data on recapture of fin-clipped fish in adjacent sections suggest only a general bias for upstream movement rather than emigration in both directions (Riley et al. 1992), although we cannot rule out this hypothesis because trout may emigrate and return between our annual sampling occasions each fall. Hunt (1971) also found that trout abundance, biomass, and production increased substantially in the control section adjacent to his treatment section, and that the effect declined in two control sections farther away. Trout population size in streams often fluctuates widely from year to year (O'Connor and Power 1976; Platts and Nelson 1988). The possibility that the population increases observed in control sections represent normal fluctuations is difficult to assess without more data, which points out the importance of long-term studies.

The experiment we report here is, to our knowledge, among the more comprehensive studies of trout population response to stream habitat enhancement done to date, incorporating measurements in treatment and control sections during pre- and post-treatment periods, spatial replication over six streams, and measurements of habitat and angler use (see Hunt 1988). Our results showed that within 2 years after installation, log drop structures caused significant increases in pool volume, mean depth, and cover, as well as increases in the abundance and biomass of adult trout. Changes in abundance may be related to increased survival of trout, but this is equivocal, and the structures caused no increases in growth or condition. We suggest that the primary function of these structures, at least during the first 2 years, was to provide more living space, which encouraged more immigrant trout to settle in treated sections.

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Appendix

Table A1. Late summer trout biomass estimates (kg·section^{-1}) for six streams in northern Colorado from 1987 through 1990.

			Age 1				Age 2 and older			
Stream	Species	Section	1987	1988	1989	1990	1987	1988	1989	1990
North Fork	Brk	CTL	0.17	0.39	0.55	0.04	3.38	2.89	3.83	4.76
			—	(0.35–0.47)	(0.49–0.66)	—	(3.29–3.52)	(2.80–3.09)	(3.31–4.45)	(4.12–5.50)
North Fork	Brk	TRT	0.21	0.27	0.92	0.13	4.18	5.80	8.17	6.84
			—	(0.24–0.31)	(0.89–0.96)	(0.13–0.15)	(3.97–4.48)	(5.40–6.29)	(7.44–8.89)	(6.76–7.02)
Colorado	Brk	CTL	0.31	0.72	0.62	1.19	7.15	4.62	5.76	7.84
			(0.30–0.32)	(0.68–0.76)	(0.59–0.65)	(1.13–1.25)	(7.05–7.25)	(4.43–4.82)	(5.58–5.94)	(7.72–7.97)
Colorado	Brk	TRT	0.70	1.02	1.20	1.28	6.95	4.18	9.44	8.87
			(0.65–0.74)	(0.94–1.10)	(1.17–1.24)	(1.22–1.34)	(6.79–7.10)	(4.13–4.26)	(9.25–9.63)	(8.57–9.17)
Walton	Brk	CTL	—	2.83	1.18	2.52	—	7.18	7.41	7.68
				(2.65–3.05)	(1.17–1.21)	(2.43–2.61)		(6.86–7.71)	(7.37–7.53)	(7.51–7.92)
Walton	Brk	TRT	—	2.25	1.41	3.78	—	5.21	5.36	8.23
				(2.19–2.32)	(1.38–1.48)	(3.65–3.91)		(5.18–5.32)	(5.28–5.54)	(8.03–8.53)
Jack	Brk	CTL	—	0.06	0.54	0.16	—	1.83	2.89	3.12
				—	(0.54–0.57)	—		(1.79–1.95)	(2.89–2.91)	(2.98–3.35)
Jack	Brk	TRT	—	0.00	0.51	0.17	—	1.21	4.75	4.24
				—	(0.45–0.61)	—		(1.17–1.33)	(4.11–5.54)	(3.90–4.63)
Beaver	Brn	CTL	0.22	0.80	1.20	0.86	8.36	7.58	8.57	10.76
			—	(0.77–0.86)	(0.91–1.83)	(0.82–0.93)	(7.71–9.02)	(7.58–7.74)	(8.32–9.09)	(10.43–11.25)
Beaver	Brn	TRT	0.21	1.67	1.81	1.16	9.21	7.05	11.74	13.49
			—	(1.44–1.96)	(1.58–2.13)	(1.16–1.19)	(8.87–9.54)	(6.48–8.05)	(11.52–12.09)	(13.49–13.61)
SSV	Brn	DS	1.42	—	0.74	2.64	4.18	—	4.83	5.87
			(1.42–1.50)		(0.71–0.77)	(2.60–2.75)	(4.18–4.27)		(4.83–4.92)	(5.87–6.07)
SSV	Brn	TRT	1.86	—	1.04	3.35	6.91	—	10.18	11.19
			(1.39–2.57)		—	(3.31–3.44)	(6.33–7.61)		(9.74–10.62)	(11.19–11.36)
SSV	Brn	US	1.44	—	0.21	1.40	2.91	—	3.72	3.23
			(1.35–1.63)		—	(1.40–1.44)	(2.91–3.09)		(3.53–4.17)	(3.23–3.69)
SSV	Brk	DS	1.20	—	0.34	0.18	1.51	—	1.66	2.01
			(1.10–1.30)		—	—	—		(1.66–1.69)	(2.01–2.07)
SSV	Brk	TRT	1.72	—	0.93	2.85	3.68	—	7.74	7.18
			(1.16–2.33)		—	(2.83–2.92)	(3.55–3.99)		(7.64–8.01)	(7.12–7.35)
SSV	Brk	US	0.90	—	0.38	1.06	1.15	—	2.31	1.83
			(0.86–0.95)		—	(1.06–1.11)	—		(1.83–3.17)	(1.83–1.94)
SSV	Rbw	DS	0.34	—	0.13	0.05	2.50	—	2.87	3.02
			—		—	—	—		(2.82–2.94)	(3.02–3.11)
SSV	Rbw	TRT	0.40	—	0.33	0.14	8.02	—	6.57	9.56
			—		—	—	(6.63–9.63)		(6.50–6.79)	(9.56–9.75)
SSV	Rbw	US	0.36	—	0.33	0.25	5.24	—	3.48	4.25
			—		—	—	(5.24–5.40)		(3.25–3.87)	(4.25–4.30)

Table A1 (*concluded*).

Stream	Species	Section	Age 1				Age 2 and older			
			1987	1988	1989	1990	1987	1988	1989	1990
SSV	Lns	DS	0.12	—	0.40	0.18	5.55	—	2.75	2.71
			—		—	—	(5.38–6.23)		—	—
SSV	Lns	TRT	0.58	—	1.46	1.55	13.91	—	6.16	4.70
			—		—	—	(11.56–16.58)		—	—
SSV	Lns	US	0.55	—	0.51	0.68	7.90	—	3.57	2.11
			—		—	—	(6.72–9.99)		—	—

Note: All sections were 250 m long, except the 375-m treatment section in S. St. Vrain Creek (SSV). Confidence limits (95%) appear in parentheses below each estimate. After 1988, the treatment section of SSV was divided into a lower and an upper section for electrofishing; estimates are combined here. Stream and species codes are as in Table 1. Lns, longnose sucker. Longnose suckers shown as age 1 were small (<205 mm); those age 2 and older were large (≥205 mm, see text).

Evidence of autumn nitrogen limitation and contribution of picoplankton to carbon fixation in Lake Tahoe

Cecily C.Y. Chang and Richard Petersen

Abstract: Water samples were collected from Lake Tahoe at midlake and nearshore stations at three depths (25, 50, and 75 m, corresponding to light intensities of approximately 10, 1, and 0.1% of surface light) in May and September. The water samples were evaluated to decide the following: (1) whether carbon fixation was N-limited and whether there were differences between picoplankton and larger phytoplankters and (2) the relative importance of picoplankton carbon fixation and related photosynthetic parameters. Evidence of N-limitation was observed only in September at the midlake station for the populations at 50 and 75 m, and to a lesser extent at the nearshore station at 75 m, but only for the nanoplankton fraction. The contribution of picoplankton to total carbon fixation ranged from 34 to 69% and did not increase with depth. Low values for I_k, the photoadaptation parameter, for deep water populations (0.1% light level) in September, but not May, suggested that these populations were adapted to low light in the fall but not the spring.

Résumé : Nous avons recueilli des échantillons d'eau du lac Tahoe à des stations situées au milieu du lac et près du rivage, à trois profondeurs (25, 50 et 75 m, ce qui correspondait à un éclairement d'environ 10, 1 et 0,1% de l'intensité lumineuse à la surface) en mai et septembre. Nous avons évalué les échantillons d'eau pour déterminer : (1) si N était un facteur limitant dans la fixation du carbone et s'il y avait des différences entre le picoplancton et les phytoplanctontes de grande taille, et (2) l'importance relative de la fixation du carbone par le picoplancton et des paramètres phytosynthétiques connexes. Des indications de la limitation par N n'ont été observées qu'en septembre à la station du milieu du lac pour les populations se trouvant à 50 et 75 m, et à un moindre degré à la station du littoral à 75 m, mais seulement pour la fraction nanoplanctonique. La contribution du picoplancton à la fixation totale du carbone se situait entre 34 et 69% et n'augmentait pas avec la profondeur. La faible valeur de I_k, le paramètre de photoadaptation, pour les populations d'eau profonde (0,1% d'éclairement) en septembre mais pas en mai peut indiquer que ces populations étaient adaptées à un faible éclairement à l'automne mais non au printemps.
[Traduit par la Rédaction]

Introduction

In oligotrophic lakes, nutrient limitation studies can be especially challenging because nutrient concentrations are near the limits of detection. This is particularly so when more than one nutrient is limiting or when limitation of a particular nutrient occurs in one season, say spring, but a different nutrient becomes limiting in the fall (seasonal switching) (Axler et al. 1991; Dodds and Priscu 1990; Morris and Lewis 1988). In a recent examination of a long-term data set, Goldman et al. (1993) reviewed the response of 110 ^{14}C bioassays (1967–1992) and concluded that Lake Tahoe shifted from N to P limitation in the late 1970s or early 1980s, consistent with the observations of other ^{14}C bioassay investigations (Arneson 1979; Goldman 1981; Byron et al. 1984; Lane and Goldman 1984; Byron and Goldman 1988; Goldman 1988). The bioassays were done with water collected from the upper 40 m of a midlake station over a 3 to 6 day period. Evidence of P limitation was demonstrated in growth (not ^{14}C) bioassays designed to test for P but not N limitation (Chang et al. 1992). These investigators collected water in the spring from the upper water column (15 m), from a nearshore and midlake station, and observed that biomass was higher in treatments that received PO_4^{-3} additions.

Primary productivity in Lake Tahoe has increased an average of 5.6% a year since 1959 (Goldman et al. 1993), but this increase has at times been higher than can be documented from direct phytoplankton counts, suggesting that

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photosynthetic picoplankton (which are not routinely included in counts) may account for a significant portion of total carbon fixation (D. Fellows, Tahoe Research Group, personal communication). The importance of picoplankton to the lake's primary productivity is not known, although pico-sized plankton are now recognized as important components of oligotrophic systems, where they comprise a higher proportion of total biomass than in meso- or eu-trophic waters (Byung and Azam 1990; Stockner 1988). There is growing evidence that the proportion of larger size plankton increases with trophic state (Gelin and Ripl 1978; Watson and Kalff 1981; Wetzel 1983; Rothhaupt and Gude 1992). There is also evidence that plankton in different size classes have different capacities for sequestering nutrients, even in mixed layers, (i.e., smaller organisms with higher affinities for a limiting nutrient will have a competitive advantage over larger organisms when nutrient concentrations are low) (Rothhaupt and Gude 1992).

Despite their potential importance, picoplankton studies in North American lakes have been limited, and only a few have provided information about primary productivity and related photosynthetic parameters e.g., I_k (the photosynthetic adaptation parameter), P_{opt} (the estimated rate of carbon uptake at optimum light intensity), and P_{max} (P_{opt}/chlorophyll *a*) (Costella et al. 1979; Fahnenstiel et al. 1986; Kalff 1972; Munawar and Fahnenstiel 1982; Vincent 1982; Stockner 1987; Fahnenstiel and Carrick 1992).

The focus of this study was to (1) determine whether primary productivity was N-limited and whether there were differences in responses to N between picoplankton (as a size class) and larger phytoplankters and (2) determine the relative contribution of picoplankton to carbon fixation and describe the photosynthetic parameters, I_k, P_{opt}, and P_{max}. Samples were collected in the spring and fall from two stations and three depths. Precautions to minimize trace metal contamination were employed (Fitzwater et al. 1982).

Methods

The nearshore Index station and deep Midlake station were described by Chang et al. (1992). Water samples were collected during the fourth year of a drought, on May 17 and 18, and Sept 13 and 14, 1990, from three depths (25, 50, and 75 m) selected to coincide with 10, 1, and 0.1% surface irradiance (Fig. 1). Three 10-L Go Flo[1] (General Oceanics) bottles cleaned with HCl using standard trace metal procedures were used (Chang et al. 1992) to collect water samples from each depth. Water samples were processed and preserved on the day of collection. Except for chlorophyll analyses (see below), three subsamples were analyzed for each depth.

Water chemistry

Fe concentration

Because Fe is an essential cofactor in nitrate reduction and can affect nitrogen availability, total Fe concentration was determined in unfiltered water by direct injection on an atomic absorption spectrophotometer (Chang et al. 1992). The detection limit was 8.5 nM and the coefficient of variation for replicate subsamples was 5%. At the Index station samples were collected from 10, 25, 50, 75 and 135 m and at the Midlake station from 25, 50, 75, 300 and 400 m. At Midlake station, surface samples were also collected with 1-L high density polyethylene bottles from the bow of a fiberglass dinghy. The dinghy was rowed some 50 m upwind from the research vessel and, using polyethylene gloves, the plastic bottles were opened beneath the surface, closed, and brought to the surface; the contents were emptied three times before collecting the final sample. The dinghy was rowed continuously upwind during sample collection. Three plastic bottles were used on each occasion. To verify that the water samples were not inadvertently contaminated by the Go Flos, all three Go Flos were used to collect samples at Midlake (25 m in May and 15 m in September) and at Index station (10 m in September).

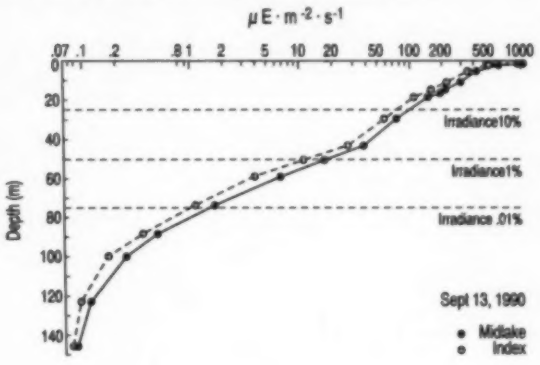


Fig. 1. Light penetration profile for Lake Tahoe.

Nutrient concentrations

The Tahoe Research Group routinely measured nutrients in the lake and supplied nutrient data (dissolved NO_3^-, NH_4^+, Kjeldahl–N and PO_4^{-3}) data for April, May, and August. Detection limits for the nitrogen analysis were 0.04 μM and for phosphorus 0.06 μM (Janik and Byron 1989). In September, samples for nutrient analysis were collected to coincide with our collection depths. As part of a cooperative agreement to evaluate quality control, September samples from 25, 50, and 75 m were split and analyzed by the Tahoe Research Group and a laboratory at the U.S. Geological Survey for dissolved NO_3^- and NH_4^+. Dissolved Kjeldahl–N was analyzed only by the Tahoe Research Group. Each laboratory analyzed two subsamples. The U.S. Geological Survey laboratory analyzed ammonium concentrations by an automated indophenol method using salicylate, where dichloroisocyanurate was used as the chlorine donor (S.W. Hager, U.S. Geological Survey, personal communication). Phosphate was measured by the method described by Ota et al. (1989), and nitrate and nitrite were measured as described by Hager and Harmon (1984). The detection limit for the nitrogen and phosphate analyses was 0.04 μM.

[1] Brand names are for identification purposes only and their use does not indicate endorsement by the U.S. Geological survey

Biological measurements

Size fractionation

For chlorophyll *a* (chl-*a*) and ^{14}C measurements, picoplankton were separated from total phytoplankton by filtering with 2-μm Nuclepore filters. Vacuum pressure for filtration did not exceed 1000 Pa.

Chlorophyll a

For chl-*a* determinations, 50 mL was filtered onto 25-mm glass fiber filters (Whatman GF/F) and buffered with two drops of saturated $MgCO_3$. Chl-*a* was determined by the acetone extraction method of Parsons et al. (1984) and analyzed fluorometrically. Two replicate subsamples were prepared for each size fraction for each sample.

^{14}C uptake

A 3.8 L sample for ^{14}C measurements was dispensed from the collection bottle from each of the three depths into linear polyethylene reservoirs enclosed in a black plastic bag to minimize exposure to light. The primary stock of $NaH^{14}CO_3$ was prepared as described by Fitzwater et al. (1982), from which a secondary stock (7.4×10^4 Bq·mL^{-1}) was prepared. One half millilitre of secondary stock was added to 42 mL of water for a final activity of 8.8×10^2 Bq·mL^{-1}. The water was prescreened through a 60-μm-mesh to remove zooplankton. Subsamples were incubated in polycarbonate centrifuge tubes (42 mL) for 2 h at each of six light intensities (193, 94, 52, 26, 16, 13, and 0 μE·m^{-2}·s^{-1}) under 'Cool White' fluorescent lights at temperatures equal to those where they were collected. Short (2 h) incubations were used to minimize the complications of declining chl-*a* and increasing abundance of heterotrophs, typical of longer incubations (Li and Dickie 1991). Six different light intensities were achieved by wrapping each of the tubes in zero to five layers of black plastic window screening or, for the dark treatment, in aluminum foil. Each size fraction and treatment had two subsamples. Samples were filtered through Millipore (GS) filters (22 mm diameter) with a nominal pore size of 0.22 μm.

The results from the ^{14}C uptake measurements were used to calculate the following photosynthetic parameters: (*i*) the initial slope of the light saturation curve, *A*, (*ii*) the rate of carbon assimilation at saturating light intensity, P_{opt}, and (*iii*) the intercept at zero light intensity, *R* (Jassby and Platt 1976) where

$$[1] \quad P = P_{opt} \times \tanh[(A \times I)/P_{opt}] - R$$

P is the rate of ^{14}C uptake (mg C·m^{-3}·h^{-1}),
I is light intensity (μE·m^{-2}·s^{-1}),
P_{opt} is the estimated rate of ^{14}C uptake at saturating light intensity (mg C·m^{-3}·h^{-1}),
A is the estimated initial slope of the light saturation curve (mg C·m^{-3}·h^{-1}·(μE·m^{-2}·s^{-1})$^{-1}$), and
R is the estimated intercept at zero light intensity (mg C·m^{-3}·h^{-1}).

This expression differs from the Jassby and Platt model in that *P*, P_{opt}, *A*, and *R* are not normalized for chlorophyll. The chlorophyll corrected parameters for carbon assimilation at optimal light intensity and the initial slope of the light saturation curve can be estimated as:

$$[2] \quad P_{max} = P_{opt}/[\text{chl}], \text{ measured as (mg C·h}^{-1}\text{·(mg chl)}^{-1}\text{)}$$

$$[3] \quad \alpha = A/[\text{chl}], \text{ measured as (mg C·h}^{-1}\text{·(mg chl)}^{-1}\text{·(μE}^{-1}\text{·m}^2\text{·s))}.$$

P_{max} is largely independent of the light intensity and spectral composition (Reynolds 1984). The value α is a measure of photosynthetic efficiency at low light intensity (Reynolds 1984). The so-called photoadaptation or light saturation parameter, I_k, (the *I* at which the initial rising part of the $P \times I$ curve intersects with P_{max}), is an indication of adaptation of the phytoplankton community to prevailing light conditions. Populations with low I_k values are presumably adapted to low light conditions. I_k can be calculated from P_{max} and α, or directly from P_{opt} and *A*:

$$[4] \quad I_k = P_{max}/\alpha, \text{ measured as (μE·m}^{-2}\text{·s}^{-1}\text{)}.$$

The ratio of picoplankton/total phytoplankton carbon fixation was calculated from the samples with the greatest ^{14}C uptake, (usually bottles with no screens).

Nitrogen limitation experiments

To test for nitrogen limitation we applied the dark ^{14}C enhancement method of Yentsch et al. (1977). The underlying premise of this method is that phytoplankton, whose growth is nitrogen limited, will show enhanced dark uptake of ^{14}C upon addition of NH_4^+ or NO_3^- (if Fe and energy supplies are not limiting) via nonphotosynthetic carboxylation reactions used in amino acid synthesis (Syrett 1956; Elrifi and Turpin 1986; Schuller et al. 1990; Vanlerberghe et al. 1990). By testing both NO_3^- and NH_4^+, we hoped to detect if there was any evidence of Fe limitation exacerbating the effects of low N availability. Populations limited by Fe and N might be expected to show enhanced ^{14}C uptake in the dark with the addition of NH_4^+ but not NO_3^-, since the lack of sufficient Fe would prevent the reduction of NO_3^- to NH_4^+.

Accordingly, 50 μL of 5 mM NH_4^+ was added as NH_4Cl to a second pair of dark tubes and 50 μL 5 of mM NO_3^- was added as $NaNO_3$ to a third pair of dark tubes, giving a final concentration of 6.0 μM nitrogen for each treatment. An additional pair of tubes was treated with 50 μL of NH_4^+ and incubated at 94 μE·m^{-2}·s^{-1} as a check for possible NH_4^+ toxicity. The addition of NH_4^+ to samples incubated in the light had only a slight effect on ^{14}C uptake and results did not suggest NH_4^+ toxicity.

Immediately after incubation, picoplankton were separated from the total phytoplankton by filtration through a 2-μm Nuclepore filter. Filtrate from the fractionated samples and unfractionated (total phytoplankton) samples were filtered through 0.22-μm Millipore filters (type GS, 25 mm) to collect all the plankton. These filters were placed directly into scintillation vials, acidified with 0.1 mL of 0.5 HCl to remove inorganic ^{14}C, and air dried prior to addition of 10 mL of fluor (Isolab Inc, solvent-free radioassay medium) and counting (Beckman LS 9000). Results of the nitrogen addition bioassays were analyzed by a two-way ANOVA. In each case, there were three depths (25, 50, 75 m) and three treatments (controls, NH_4^+, and NO_3^-).

Results

Water chemistry

In general, Fe concentrations showed little variation with depth below 75 m (Fig. 2). Maximum concentrations at Index (10 m) and Midlake (25 m) stations were three and two times higher, respectively, than bottom concentrations. In May, concentrations tended to decrease with depth, with a possible loss of Fe in near surface waters (10 m).

By September, surface concentrations of Fe had decreased and were lower than in the near surface waters. Iron concentrations were higher and more variable in the upper 50 m than in May. Similar concentrations among the replicated samples (surface samples, May Midlake (25 m) and September Index (10m)) suggest that collection protocol and analytical procedures were consistent.

Dissolved NO_3^- and NH_4^+ concentrations in Lake Tahoe were low, and rarely exceeded 0.50 μM in the euphotic zone except immediately after lake mixing (Fig. 3A). Typical nutrient profiles for NO_3^- and NH_4^+ showed a nitracline (NO_3^-) gradient between 50 and 75 m (Fig. 3A, 3C). Midlake September NO_3^- concentrations (Fig. 3B) agreed well with those for May–August (Fig. 3a). Nitrate concentrations increased with depth, and reached 2.84 μM toward the lake bottom. Ammonium concentrations in the upper 100 m typically ranged from 0.1 to 0.2 μM, near the detection limit, and were low throughout the water column. Differences in NO_3^- and NH_4^+ concentrations between subsamples analyzed by the two labs (25, 50 and 75 m September samples) were not unexpected, given that concentrations were near the detection limit (Fig. 3B, 3D). Dissolved Kjeldahl was approximately 4.0 μM at both stations, an order of magnitude higher than NO_3^- concentrations, and showed no variation with depth.

Spring and summer total P concentrations in Lake Tahoe range from 0.06 to 2 μM with dissolved concentrations ranging from below the detection limit (0.06 μM) to 0.40 μM, making it difficult to resolve the structure of the phosphorus profile with depth (Goldman 1988). Soluble reactive phosphorus was near the detection limit (0.06 μM) (data not shown).

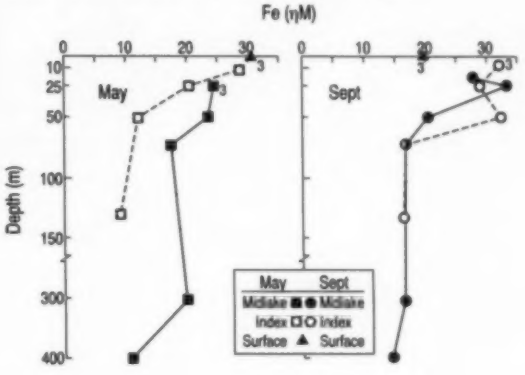
Chl-*a*

Total chl-*a* concentrations in May decreased with depth at the Index station but increased at Midlake (Table 1). In contrast, total chl-*a* concentrations in September increased with depth at both stations. Chl-*a* concentrations at the Index station were higher than at the Midlake station in September. Picoplankton accounted for 20–50% of total chl-*a* in May and was greatest at 25 m at Midlake, whereas at the Index station the greatest contribution of picoplankton chl-*a* occurred at 75 m. In September picoplankton accounted for ≥80% of chl-*a* at Midlake and 27–60% of the total chl-*a* at the Index station.

Primary productivity

Values obtained for α, P_{max}, and I_k are generally consistent with values reported elsewhere, which range from 0.007 to 0.133 for α, from 20 to 500 for I_k, and from 0.5 to 20 for P_{max} (Kirk 1983; Reynolds 1984; Fee et al. 1987) (Table 1). In May, P_{max} and I_k were greatest for populations

Fig. 2. Total iron (Fe) concentrations. Midlake surface concentrations sampled from the dingy are depicted by triangles. Hydrocast samples are indicated by circles and squares. Detection limit for Fe was 8.5 nM and the average CV of 10% represents analytical error.



at the 0.1% (75 m) light level, a result of elevated P_{opt} rates. That the deep populations had elevated I_k values is surprising, since these populations receive less light than those at the surface, and would be expected to have low I_k values. Though this may arise because of isothermal conditions that in turn would result in a well-mixed water column without distinct phytoplankton populations as a function of depth, temperature and NO_3^- profiles showed that the water column was temperature stratified in May. In general, α values were higher at Index than at Midlake.

In September the consistent decrease in I_k with depth showed that the phytoplankton were adjusting to prevailing light conditions.

The ratios of picoplankton/total phytoplankton carbon fixation listed under the heading '% pico C', ranged from 23 to 69% and showed no increase with depth (Table 1) in contrast to observations by others (Costella et al. 1979; Li et al. 1983; Platt et al. 1983; Craig 1984; Glover et al. 1985*a*, 1985*b*).

The photosynthetic parameters were used to estimate the range of in situ primary productivity (400–800 mg $C \cdot m^{-2} \cdot day^{-1}$) in Lake Tahoe (Table 2). This range is slightly higher but in reasonable agreement with rates reported by the Tahoe Research Group (TRG) for the Index station during 1983–1987 (330–400 mg $C \cdot m^{-2} \cdot day^{-1}$ in May and 250–600 mg $C \cdot m^{-2} \cdot day^{-1}$ in September). It is also well within the range of values reported for oligotrophic and mesotrophic lakes (Wetzel 1983) including Crater Lake (C.C.Y. Chang and R. Petersen, unpublished).

Nitrogen limitation

In May, addition of NH_4^+ to the bottles did not cause significant change in the uptake of ^{14}C in either the total plankton or picoplankton (Table 3). This suggests that nitrogen was not limiting. Analysis of variance also showed that there was no interaction between depth and the addition of nitrogen (Table 4).

In September, however, carbon fixation increased in the deep water populations that received either NH_4^+ or NO_3^-,

Table 1. Photosynthetic parameters determined from ^{14}C measurements.

Depth (m)	*Y* intercept	P_{opt} (mg C·m^{-3}·h^{-1})	P_{max}	*A* slope	α	I_k	Chl (mg C·m^{-3})	% pico C[a]
			Midlake station May					
Total phytoplankton								
25	1.22	0.79	1.93	0.019	0.046	42	0.41	—
50	1.27	—	—	0.014	0.027	—	0.52	—
75	1.43	14.19	10.59	0.060	0.045	236	1.34	—
			Index station May					
25	2.21	1.07	0.91	0.034	0.029	31	1.17	—
50	1.57	2.15	3.71	0.064	0.111	33	0.58	—
75	1.21	2.28	6.00	0.041	0.107	56	0.38	—
			Midlake station May					
Picoplankton								
25	1.22	0.34	1.62	0.009	0.043	38	0.21	42
50	1.22	0.35	1.84	0.018	0.095	20	0.19	31
75	1.43	0.71	1.50	0.017	0.036	40	0.47	41
			Index station May					
25	1.23	1.21	4.65	0.082	0.316	15	0.26	29
50	1.02	1.21	7.12	0.128	0.753	9	0.17	56
75	1.21	2.28	12.00	0.041	0.216	56	0.19	25
			Midlake station September					
Total phytoplankton								
25	1.38	1.04	11.56	0.008	0.088	125	0.09	—
50	1.30	1.10	9.16	0.017	0.142	64	0.12	—
75	1.32	1.21	3.67	0.021	0.064	56	0.33	—
			Index station September					
25	1.34	1.18	4.21	0.011	0.040	106	0.28	—
50	1.34	1.13	1.67	0.011	0.016	106	0.68	—
75	1.21	2.41	2.13	0.063	0.056	38	1.13	—
			Midlake station September					
Picoplankton								
25	—	0.29	2.42	—	—	—	0.12	34
50	1.42	0.41	2.93	0.007	0.050	57	0.14	44
75	1.29	0.54	2.00	0.012	0.044	46	0.27	46
			Index station May					
25	1.24	0.70	4.12	0.012	0.072	59	0.17	54
50	1.28	0.84	7.00	0.007	0.058	115	0.12	69
75	1.28	0.55	2.89	0.020	0.110	27	0.19	23

Note: Units for P_{max} are mg C·h^{-1} (mg chl)$^{-1}$; for α, mg C (mg chl·h)$^{-1}$·(μE^{-1}·m^{-2}·s), and for I_k, μE·m^{-2}·s^{-1}.
[a]Ratio of picoplankton to total phytoplankton carbon fixation.

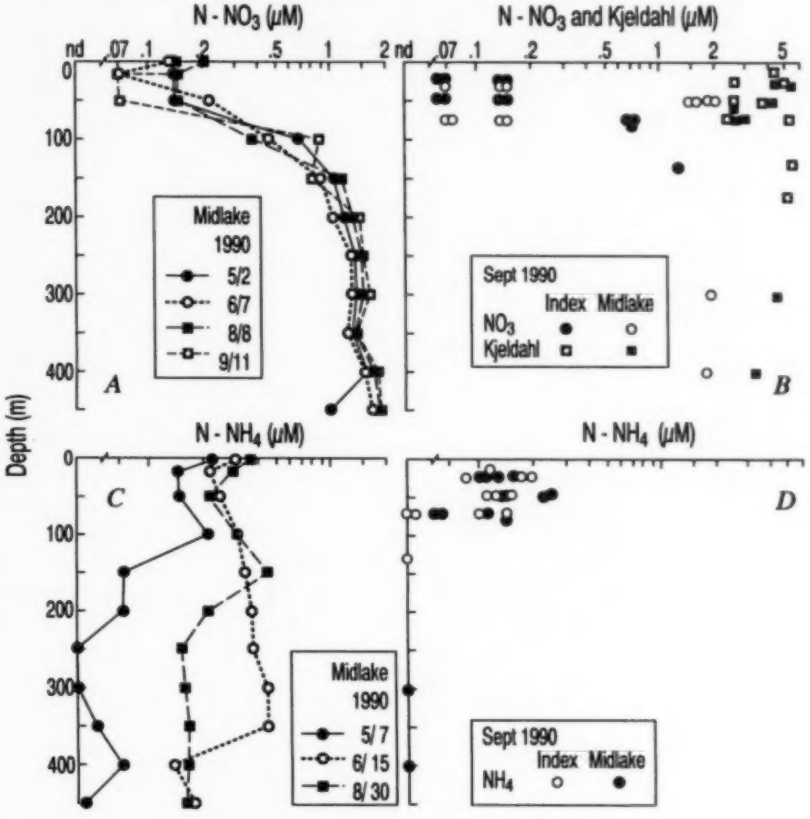
suggesting N limitation (Table 3). One response of nitrogen-limited populations is to show dark enhancement of carbon fixation (Yentsch et al. 1977) upon the addition of nitrogen, and depressed rates in the light (Flynn 1990). Analysis of variance also showed that there were significant effects of depth and nitrogen addition at the Midlake station for both total plankton and picoplankton (Table 4). This suggests that nitrogen limitation at the Midlake station was more pronounced with depth in contrast to results at the Index station, where there was only a significant depth effect.

Under light conditions, the September Midlake phytoplankton populations at 25 m exhibited enhanced carbon fixation, with NH_4^+ : control ratios of 1.58 and 1.44 for picoplankton and total samples respectively. Otherwise, light bottles showed no response to NH_4^+ additions.

Discussion

Results from this study show that picoplankton in Lake Tahoe comprise a substantial portion of total chl-*a*. In

Fig. 3. Dissolved nitrogen concentrations at Lake Tahoe (note break in *x* axis and log scales). (A) typical N–NO_3 concentrations. (B) September N–NO_3 and N–Kjeldahl concentrations for each of the replicates. (C) Typical N–NH_4 concentrations. (D) September N–NH_4 concentrations for each of the replicates. Data for A and C provided by the Tahoe Research Group.



September chlorophyll concentrations increased with depth and I_K decreased with depth. These results are consistent with the water chemistry data that suggest that summer stratification led to a corresponding stratification of the plankton community.

The calculated photosynthetic parameters for Lake Tahoe did not always coincide with the observations of Platt et al. (1983) for the North Atlantic, where P_{max}, and the initial slope, α, were higher for picoplankton. They also observed that I_k was lower for picoplankton than for phytoplankton greater than 1 μm. Pico A was greater than total A in this study only in May at Midlake 50 m, and at Index at 25 and 50 m (Table 1). Furthermore, pico α was greater than total α in May at 50 m for both stations and in September at 75 m Midlake only. Likewise, pico I_k < total I_k for only the following samples: in May at 25 and 75 m at Midlake, and 25 and 50 m at Index; in September, at 50 and 75 m at Midlake and 75 m at Index.

It is not surprising that our estimates of primary productivity are different from the values reported by the TRG, given that a different protocol was used. For example, TRG incubates samples in situ, rather than in an incubator as used in this study, and uses 0.4-μm pore size filters to recover phytoplankton for ^{14}C counts. This leaves open the possibility that some of the pico–plankton were not retained. We estimate that picoplankton accounted for

Table 2. Estimated range of primary productivity values for Lake Tahoe.

Date and station	mg C·m^{-2}·day^{-1} Total	Picoplankton
May		
Index	662	789[a]
Midlake	371	196
September		
Index	583	419
Midlake	549	187

[a]Because P_{opt} total < P_{opt} picoplankton for 25-m sample. See *P* vs *I* parameters.

Table 3. Effects of NH_4^+ and NO_3-additions on ^{14}C uptake in the dark.

	Control		NH_4^+ addition		NO_3^- addition		NH_4^+/control		NO_3^-/control	
Depth (m)	Pico	Total	Pico	Total	Pico	Total	Pico	Total	Pico	Total
Midlake station May										
25	31.2	29.8	29.5[a]	35.4[a]	—	—	0.95[a]	1.19[a]	—	—
50	31.5	32.6	31.3	29.8	—	—	0.99	0.91	—	—
75	29.7	31.5	30.5	30.6	—	—	1.03	0.97	—	—
Index station May										
25	33.2	30.6	32.0	30.5	—	—	0.96	1.00	—	—
50	31.0	32.5	32.8	30.0	—	—	1.06	0.92	—	—
75	30.7	31.7	30.2	32.4	—	—	0.98	1.02	—	—
Midlake station September										
25	34.0	30.3	30.8	31.2	33.3	33.4	0.91	1.03	0.98	1.10
50	42.8	33.5[a]	104.1	103.2	119.8	119.6	2.43	3.08[a]	2.80	3.57
75	32.8	35.2	155.2	171.9	161.5[a]	157.1[a]	4.73	4.88	4.92[a]	4.46[a]
Index station September										
25	32.2	33.2	29.0	32.2	34.2	34.8	0.90	0.97	1.06	1.04
50	38.1	36.4	44.8	60.7	57.4	79.5	1.18	1.67	1.51	2.18
75	32.9	33.8	70.2	60.4[a]	60.2	83.5[a]	2.13	1.79[a]	1.83	2.47[a]

Note: Numbers in each column represent the mean of two subsamples in counts per minute (cpm).
[a]Values are based on one sample only.

Table 4. Results of two-way ANOVA for September N treatments (NH_4 and NO_3) in the dark.

Source of variation	Index station		Midlake station	
	Total plankton	Picoplankton	Total plankton	Picoplankton
Nitrogen	NS	NS	**	**
Depth	*	*	**	**
Interaction	NS	NS	*	NS

$^{**}P < 0.01$, $^{*}P < 0.05$, NS > 0.05.

34–72% of in situ primary productivity and suggest that picoplankton are an important component of the phytoplankton throughout the water column.

Nitrogen limitation

Phytoplankton response to nitrogen addition suggests that phytoplankton collected at 75 m were N-limited in September but not in May, which was not expected. Dissolved nitrogen tends to be depleted in the upper 75 m of the water column in the fall. At 75 m, dissolved NO_3^- was between 0.07 and 0.15 μm, while NH_4^+ concentration was somewhat less, near the detection limit. At the Midlake station, all phytoplankton including picoplankton were N-limited at 50 and 75 m in September, whereas at the Index station, only the nanoplankton appeared limited at these depths. The higher NH_4^+ : control ratios for the Midlake samples (Table 2) also suggest that limitation was more acute at the Midlake station than at the Index station. While a shortage of Fe may inhibit reduction of NO_3^- to NH_4^+, our short-term bioassays showed no difference between NH_4^+ and NO_3^- addition, suggesting that there was sufficient Fe (10–30 nM) and insufficient nitrogen. These results are not definitive regarding the question of Fe limitation, because short-term dark ^{14}C bioassays occasionally give inconsistent results when applied to systems with more than one limiting nutrient, e.g., N and P (Dodds and Priscu 1990).

Phosphate limitation was demonstrated by Chang et al. (1992) when incremental additions of phosphate to lake water resulted in an increase in phytoplankton biomass (2-week bioassays). The long-term data set of Goldman et al. (1993) also suggests that the lake is primarily P-limited. However, this result is based on bioassays (3–6 days) performed on samples collected from 0 to 40 m. Our results indicated nitrogen limitation only for samples collected from deeper in the lake.

To conclude, our results suggest that the deep water nanoplankton population in Lake Tahoe was N-limited and

adapted to low light in September but not in May, and that picoplankton accounted for 34–69% of primary productivity.

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From picoplankton to microplankton: temperature-driven filtration by the unionid bivalve *Lampsilis radiata siliquoidea* in Lake St. Clair

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Abstract: Size-selective filtration by the unionid bivalve *Lampsilis radiata siliquoidea* was determined seasonally for Lake St. Clair seston with special emphasis placed on determining retention efficiency of particles less than 1 μm equivalent spherical diameter (ESD). These results allowed us to estimate the aggregate clearance rates of *Lampsilis* and total unionids in the lake. Retention efficiency for seston in the 0.79–1.00 μm ESD size category was 0.44 whereas 0.07 was the corresponding value for 0.91-μm microspheres in laboratory prepared mixtures of microspheres and cultured algae; respective values for the 0.63–0.79 μm size category were not significantly different from zero. Particle shape may explain the difference between natural seston and microspheres. Particles in the picoplankton size range (<3 μm ESD) were probably an important food resource to the mussels, since 35–49% of the seston volume in Lake St. Clair was smaller than 3 μm ESD. Regression analyses of filtration rate as a function of temperature and food concentration showed that feeding rate was temperature driven and not saturated by available seston concentration. *Lampsilis* and total community clearance rates were maximum in September, with respective fractions of the water columns swept clear of 0.018 and 0.053·d^{-1}.

Résumé : Nous avons mesuré de façon saisonnière la granulométrie de la filtration du seston du lac Ste-Claire par le bivalve unionidé *Lampsilis radiata siliquoidea*, et particulièrement l'efficacité de la rétention des particules de moins de 1 μm de diamètre équivalent sphère (ESD). Ces résultats nous ont permis d'estimer les taux globaux de filtration de *Lampsilis* et de l'ensemble des unionidés du lac. L'efficacité de la rétention pour la seston de la catégorie de taille 0,79–1,00 μm ESD était de 0,44, contre 0,07 pour des microsphères de 0,91 μm dans des préparations de laboratoire contenant un mélange de microsphères et d'algues cultivées; les valeurs respectives pour la catégorie de taille 0,63–0,79 μm n'étaient pas significativement différentes de zéro. La forme des particules peut expliquer la différence entre le seston naturel et les microsphères. Les particules situées dans la plage de taille du picoplancton (<3 μm ESD) constituaient probablement une source de nourriture importante pour les bivalves, car 35–49 % du volume du seston dans le lac Ste-Claire était de taille inférieure à 3 μm ESD. Les analyses de régression du taux de filtration en fonction de la température et de la concentration de nourriture ont révélé que le rythme d'alimentation était régi par la température et non saturé par la concentration du seston disponible. Les taux de filtration de *Lampsilis* et de l'ensemble de la communauté étaient au maximum en septembre, les fractions filtrées de la colonne d'eau étant respectivement de 0,018 et 0,053·d^{-1}.
[Traduit par la Rédaction]

Introduction

Relatively little work has been done on the filtration rates and particle retention efficiency of unionid mussels, especially on natural seston (e.g., Paterson 1984; Kryger and Riisgard 1988). Filtration rate and particle retention efficiency of *Lampsilis radiata siliquoidea* were determined seasonally at monthly intervals on natural seston as part of a larger project to understand the role of mussels in particle and nutrient cycling in Lake St. Clair (Nalepa et al. 1991a). *Lampsilis r. siliquoidea* was the dominant mussel, accounting for 45% of the biomass (Nalepa and Gauvin 1988) until the precipitous decline of all native mussels following the invasion by the European mussel *Dreissena polymorpha* (Nalepa 1994). *Lampsilis r. siliquoidea* was also the dominant native unionid in Lake Erie (Nalepa et al. 1991b). By measuring these filtering rates and combining them

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Table 1. Experimental variables in tub experiments with *L. r. siliquoidea*: tub volume (V), duration of experiment (t), number (N) of mussels per tub, dry weight of mussels expressed as range for all mussels in experiment and mean (±SE) of mean weights of mussels in each tub, and particle size range examined by the Coulter counter expressed as ESD.

Date	V(L)	N	t(h)	Dry wt (g) Range	Dry wt (g) $\bar{X}$ ± SE	ESD range analyzed (μm)
Sept. 15, 1986	4.0	5–6	1.6	0.82–3.15	1.61 ± 0.18	2.0–40.0
Oct. 15, 1986	7.0	3	1.5	0.80–2.95	1.78 ± 0.10	0.63–80.6
Apr. 21, 1987	7.0	3	1.5	0.99–3.16	1.92 ± 0.16	2.0–40.0
May 20, 1987	7.0	3	1.7	1.25–3.04	2.13 ± 0.12	1.0–80.6
June 30, 1987	7.0	3	2.0	1.56–2.64	1.98 ± 0.10	2.0–40.0
Aug. 5, 1987	7.0	3	1.1	1.26–2.89	1.91 ± 0.08	1.0–80.6
Sept. 9, 1987	7.0	3	1.0	0.93–2.11	1.59 ± 0.08	0.63–40.0
Sept. 10, 1987[a]	7.0	3	1.0	0.93–2.11	1.59 ± 0.08	0.63–40.0
Nov. 19, 1987	3.0	1	2.0	1.01–1.94	1.52 ± 0.22	2.0–40.0

[a]Experiment with *Chlamydomonas* and microspheres.

with biomass estimates of *Lampsilis* for Lake St. Clair (Nalepa and Gauvin 1988), we estimated the aggregate filtering impact of *Lampsilis* and the total unionid community on suspended particles in Lake St. Clair.

We were particularly interested in defining particle retention efficiency down to the smallest particles retained and the role of environmental variables on filtration rate. Few reliable data are available for filtering rates of unionids (Kryger and Riisgard 1988), and there are no comprehensive seasonal studies with natural seston for any bivalve. Moreover, to our knowledge, small-particle (<4 μm) retention efficiency has not been measured for unionids, and no retention efficiencies are available for any freshwater or marine bivalve for seston below 1 μm (e.g., Mohlenberg and Riisgard 1978; Jorgensen et al. 1984; Paterson 1984; Jorgensen 1990). Significant retention efficiencies below 1 μm equivalent spherical diameter (ESD) are to be expected because many marine bivalves showed retention efficiencies on the order of 50% for natural seston of 1.1 μm ESD (Mohlenberg and Riisgard 1978; Jorgensen et al. 1984). Until retention efficiency of small particles is defined, we cannot understand impact of bivalve filtering on aquatic communities, which contain a significant portion of biomass and production in the picoplankton (e.g., Fahnenstiel and Carrick 1992), we cannot understand competitive interactions between bivalves, and we cannot relate filter performance to filter design (e.g., Jorgensen 1990).

We suspect that the lack of measurements below 1 μm ESD relates to the high skill level required to make these measurements with the Coulter counter. We were able to draw on our experience (e.g., Vanderploeg 1981a, 1981b) to delineate the entire retention efficiency versus ESD curve that extends from the microplankton down to particles in the size range of picoplankton, where picoplankton and microplankton size ranges are defined respectively as <3 μm (Fahnenstiel and Carrick 1992) and 20–200 μm (Wotton 1990).

Methods

General experimental design

To determine filtering rates of *Lampsilis* on natural seston, we used the clearance, or tub, method in which a number of freshly collected mussels were confined in experimental tubs with freshly collected lake water and two tubs of the same size as the experimental tubs without mussels served as controls (Table 1). Clearance rate (F_i) on particle size category i was calculated from the relation $F_i = \ln(C_i/Z_i)V/nt$ (e.g., Vanderploeg and Scavia 1979), where C_i = mean concentration of particles in size category i in control containers at end of experiment, Z_i = concentration of particles in size category i in an experimental container at end of the experiment, V = tub volume, n = number of mussels, and t = duration of experiment.

Particle retention efficiency was determined by both clearance and siphon (suction) methods (Mohlenberg and Riisgard 1978; Kryger and Riisgard 1988). For the clearance method, retention efficiency is identical to the selectivity coefficient W_i' (Vanderploeg and Scavia 1979; Vanderploeg 1990): $W_i' = F_i/F_{pref}$, where F_{pref} is the clearance rate of the preferred size category. Assuming the preferred particles are filtered with 100% efficiency, F_{pref} represents the rate at which water is pumped through the bivalves's filter, i.e., the "filtration rate". In the siphon method (Fig. 1), water near a mussel's inhalant siphon was sampled simultaneously with water leaving the exhalant siphon (Mohlenberg and Riisgard 1978): the fraction of particles in size category i retained (P_i) by the bivalve was $1 - (E_i/I_i)$, where E_i = concentration of particles in size category i in the exhalant siphon and I_i = simultaneous concentration at inhalant siphon; retention efficiency (W') was calculated as $W_i' = P_i/P_{pref}$, where P_{pref} is the fraction of particles retained in the preferred size category.

In terms of particle selection, both tub and siphon methods give estimates of what is filtered; they do not provide information on particle selection subsequent to particle

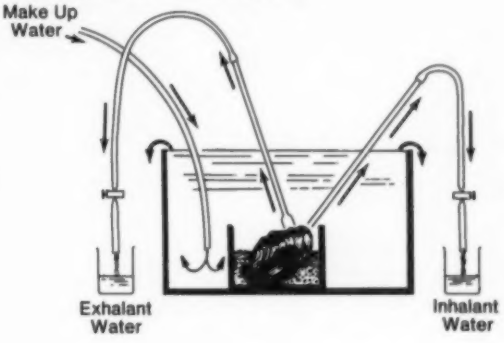
filtration, if such selection occurs. Rejected particles appearing in mucus-coated pseudofeces are expelled through the inhalant siphon back into the experimental suspension and tend to sink to the bottom. Feces are deposited on the bottom. Both kinds of these mussel-produced particles could show up in significant quantities in the experimental suspension if this particle production is high and vigorous stirring resuspends them. In the tub method, resuspended produced particles could reduce estimates of filtering rates in those size categories of the same size as the produced particle, and hence reduce estimates of retention efficiency in those size categories. High production of pseudofeces and feces would only be expected for satiating concentrations of food or poor quality of food.

Because only particles actually leaving the mussel's exhalant siphon are sampled in the siphon method, effect of particle production is minimized in the siphon technique (Mohlenberg and Riisgard 1978). This theoretical advantage was the reason we went to the extra effort (see below) of using this method.

In addition to measuring particle retention on natural seston, we used carboxylated microspheres (Polysciences Inc.) mixed with algae. Both organic and inorganic seston are variable in shape. By using spheres, we eliminated the variable of particle shape. Carboxylated spheres were chosen because (1) their negative charge kept them from clumping and (2) natural particles in lake water have a negative charge (Gerritsen and Bradley 1987).

Use of the tub method for estimating filtration rates has recently been questioned by Kryger and Riisgard (1988), who reported a much higher filtration rate for bivalves with the siphon method than earlier investigators had reported for the tub (and other) methods. They attributed the difference to two possibilities: (1) earlier investigators used high particle concentrations and particles of poor food quality and (2) mussels buried in sediment, in the siphon method, have intrinsically higher filtration rates than mussels lying on their sides without sediment, as in the tub method, because mussels lying on one valve do not fully open their valves and siphons. We evaluated the second hypothesis by comparing filtering rates of mussels first buried in sand and subsequently lying on one valve without sand.

Fig. 1. Suction (siphon) apparatus, modified from that of Mohlenberg and Riisgard (1978), used in the mussel feeding experiments. Water is sampled near the inhalent siphon and particle concentration compared with that in water leaving the exhalent siphon.



Collection

Lampsilis were collected using an epibenthic sled in western Lake St. Clair at station 73 (42°30′N, 82°50′W) (Nalepa and Gauvin 1988), a site having a sandy silt substrate. Sampling occurred from September 1986 to November 1987 at approximately monthly intervals during spring through fall for estimates of seasonal filtering rate (Table 1) and on two later occasions (June 1988 and September 1992) for siphon experiments and comparison of siphon and clearance methods. All mussels used for experiments did not have any attached *D. polymorpha* when collected. The mussels used in the experiments were representative of the 9- to 12-yr-old mussels that dominated the population in the lake (Nalepa and Gauvin 1988): the range of mean mussel dry weights (1.5–2.1 g) in the experiments corresponded to 9- to 11-yr-old mussels. Water used for the feeding experiments came from the same station, collected with a Van Dorn water bottle 1 m off the bottom. Upon collection, mussels were placed in insulated coolers with lake water for transport to our laboratory in Ann Arbor, Michigan, where they were placed in an environmentally controlled room in dim light set to ambient lake temperatures. Lake water to be used for feeding experiments was transported in 11-L insulated picnic jugs. Shells of mussels were gently cleaned of adhering debris.

Laboratory methods

Seston concentration was measured as chlorophyll *a* determined fluorometrically (Strickland and Parsons 1968) and as particle volume measured with a TA II Coulter counter using the multitube technique (Vanderploeg 1981a, 1981b). The volumetric units of the Coulter counter, expressed as cubic millimetres per litre, or parts per million by volume, correspond to milligrams per litre if density of the seston is assumed to be 1. Whenever possible, we used a combination of 30-, 100-, and 200-μm-aperture tubes to characterize the particle size spectrum between 0.63 and 80 μm ESD. The 30-μm-aperture tube was difficult to use with seston because of problems with particle plugging and sensitivity to electrical noise; therefore, we often instead had to use 50, 70, or a 100 μm as the smallest tube aperture, which allowed measurement of particle ESDs as small as 1.0, 1.6, and 2.0 μm, respectively. Lake water was made conductive by adding enough 0.2-μm-filtered 25% NaCl solution to make a final solution of 0.5 or 1.0% NaCl. Precision of the Coulter counter for concentration of small particles, which are numerically abundant and the primary focus of our study, was better than ±2%. Precision of the less abundant large particles was related to number counted as defined by Poisson statistics, and statistical treatment of the data followed that of Vanderploeg (1981b).

Chlamydomonas sp. and *C. oblonga* were cultured by methods described by Vanderploeg et al. (1984). Dry weights of soft tissue were obtained for all mussels after drying at 60°C for at least 48 h.

Tub experiments

All eight tub experiments using natural lake seston were conducted on the day of collection. The one tub experiment with microspheres (September 10, 1987) was done the day after collection using mussels from the seston feeding experiment of the day before (Table 1); the feeding suspension was 0.74-μm (SD = 0.009) carboxylated microspheres (0.5 $mm^3 \cdot L^{-1}$) and *Chlamydomonas* sp. (0.85 $mm^3 \cdot L^{-1}$) and *C. oblonga* (1 $mm^3 \cdot L^{-1}$) in 0.2-μm-filtered lake water (Table 1).

To set up a tub experiment, water was dispersed among six tubs of identical size (3, 4, or 7 L) from a continually mixed 50-L carboy with spigot. To ensure similar particle-size spectra in all control (C1, C2) and experimental (Z1–Z4) tubs, all tubs were filled one quarter full in the order C1, Z1, Z2, Z3, Z4, and C2 and the filling in one quarter parts was repeated until all tubs were full. Subsamples taken immediately from C1 and C2 were used for estimating initial concentrations. After the first experiment, we decided that, when available, three mussels per 7 L provided reasonable numbers of mussels for purposes of replication, and for removal of a sufficient fraction of the seston during the 1- to 2-h experimental feeding period for calculation of accurate filtering rates (Table 1). During the experiment, the tubs were gently stirred (without disturbing the mussels) for about 10 s at 7- to 10-min intervals to keep particles in suspension while minimizing stirring up of pseudofeces or feces that might have been produced by the mussels.

Siphon (suction) experiments

Siphon experiments (Fig. 1) were conducted using a method almost identical to that of Mohlenberg and Riisgard (1978). A mussel was first put into a plastic dish (9 cm diameter × 6 cm high) filled with washed sea sand. This was placed into an 8-L tub containing the feeding suspension. The mussel was then left overnight to bury itself in the sand. We often assisted burying by placing mussels in a shallow depression in the sand. On the next morning, two glass sampling tubes were fixed into position, one at the inhalent siphon and one at the exhalent siphon. Latex tubing was attached to the glass tubes to draw off the water samples by gravity into small beakers. Flow rate in both sample tubes was adjusted with screw clamps to be slower than the flow rate of the exhalent siphon to ensure that the exhalent water sample came only from the exhalent siphon and not the surrounding water. The feeding suspension was continually replenished from a reservoir above the experimental tub. The inflow was slightly faster than the outflow so that the tub overflowed, maintaining a constant 8-L volume during the experiment. The feeding suspension was either lake seston or a mixture of 1 $mm^3 \cdot L^{-1}$ each of 0.74 (SD = 0.009) and 0.91-μm (SD = 0.03) carboxylated microspheres and 2 mm^3 *C. oblonga*$\cdot L^{-1}$ suspended in 0.2-μm-filtered lake water.

Comparison of mussels in and out of sediment

Mussels were collected on September 3, 1992 and, on the same day of collection, each mussel was placed in its own sand-filled dish (described above) inside of its own 5-L tub of 35-μm-filtered lake water under dim light at ambient conditions of a 14 h light : 10 h dark cycle at 20°C. Feeding experiments were initiated the next afternoon with mussels buried in the sand with their siphons pointing upward (Fig. 1). *Chlamydomonas oblonga* was added to the tubs to make a concentration of 2 $mm^3 \cdot L^{-1}$ because the mussels eliminated nearly all the particles in the tubs overnight. *Chlamydomonas oblonga* was an ideal alga for this purpose because it is large enough to be retained with 100% efficiency and experiments showed that its small size and motility keep it from sinking out. Water samples (75 mL) for calculation of clearance rates were collected at intervals between 0.5 and 1 h from all tubs. Clearance rate was calculated from the same formula as given above for the tub method, except that initial concentration of an interval replaced control concentration in the formula. The containers were gently stirred every 15 min to ensure a homogeneous suspension. Clearance rates for the mussels in sand reported here are those taken after the mussels were allowed to feed for an hour to be sure they were fully acclimated (as evidenced by full siphon opening) to the experimental conditions of the introduced *Chlamydomonas*.

To obtain comparative rates for the mussels lying on one valve, mussels were removed from their dishes (without taking them out of water) and placed on their sides in the same tubs. To ensure that approximately comparable food concentrations were available to mussels in and out of sand, we added enough *Chlamydomonas* to increase the concentration remaining after feeding in the sand by 1 $mm^3 \cdot L^{-1}$. An interval of 15–30 min elapsed before water samples were taken for initial particle concentration. After about 1 h, final particle concentrations were taken for estimation of clearance rates. Similar experiments were repeated with the same mussels on September 9 and 10. Between experiments, mussels were kept in good condition by occasional changes of the water and feeding with commercial oyster food (preserved, concentrated *Thalassiosira pseudonana*).

Results

Particle-retention efficiency

Natural seston

Tub and siphon experiments showed that the mussels filtered natural seston with nearly 100% efficiency once particles become as large as 1.3–3 μm (Table 2). Submicrometre particles in the 0.79–1.00 μm ESD category were filtered with a relatively high W' of 0.44–0.46, whereas particles in the 0.63–0.79 μm ESD category had a very low W' of 0.01, which was not significantly ($P > 0.05$) different from 0. Although a broad size spectrum of particles (Table 1; Fig. 2 and 3) was analyzed in the experiments, W' data in Table 2 were truncated above 4 μm because the W' versus ESD curves flattened out by 4 μm. One experiment, the tub experiment of April 21, 1987, is omitted from Table 2 because there was significant particle production that resulted in higher particle concentrations in experimental tubs than in control tubs over the size range from 2 to 18 μm. We do not know the cause of this addition, but speculate that the mussels had just emerged from their deep winter burial and had not adjusted to normal filtration.

Table 2. Mean retention efficiency (W') of *L. r. siliquoidea* feeding on natural seston as a function of equivalent spherical diameter (ESD) of particles. W' values were calculated from filtering rates restricted to 0.63–4.00 μm ESD size categories.

Experiment	ESD of lower boundary of size category (μm)[a]								
	0.63	0.79	1.00	1.26	1.59	2.00	2.52	3.17	4.00
Tub									
Sept. 15, 1986						1.00	0.95	0.86	0.73
Oct. 15, 1986	0.00	0.46	0.75	1.00	0.96	0.96	0.96	0.96	0.98
May 20, 1987			0.49	0.79	0.87	0.88	0.90	0.88	1.00
June 30, 1987						0.91	1.00	0.90	0.81
Aug. 5, 1987			0.80	0.92	0.97	1.00	0.96	0.85	0.85
Sept. 9, 1987	0.03	0.40	0.75	0.93	0.96	0.94	0.99	1.00	0.97
Nov. 19, 1987						0.89	0.98	0.99	1.00
Mean	0.02	0.43	0.70	0.91	0.94	0.94	0.96	0.92	0.90
SE	0.02	0.03	0.07	0.04	0.02	0.02	0.01	0.02	0.04
Siphon (five mussels)									
June 29, 1986	0.00	0.46	0.78	0.91	0.94	0.97	0.97	1.00	0.97
Tub and siphon									
Mean	0.01	0.44	0.71	0.91	0.94	0.94	0.96	0.93	0.91
SE	0.01	0.02	0.06	0.04	0.02	0.02	0.01	0.02	0.04
Tub (Oct. 15, 1986, and Sept. 9, 1987) and siphon (June 29, 1988)									
Mean	0.01	0.44	0.76	0.95	0.95	0.96	0.98	0.99	0.97
SE	0.01	0.02	0.01	0.03	0.01	0.01	0.01	0.01	0.004

[a]The upper boundary of a size category is the lower window of next larger size category; for example, the 0.63-μm ESD size category includes particles in the size range 0.63–0.79 μm.

The mean W' values of respective channels of the tub experiments and siphon experiments with natural seston were not appreciably different and in most channels not significantly ($P > 0.05$) different (Table 2); therefore, we determined an overall mean calculated from the mean of the tub experiments and the mean result for the siphon experiment. This overall mean for each channel was calculated in two ways for Table 2: (1) the siphon mean was averaged with the respective mean calculated from all tub experiments and (2) the siphon mean was averaged only with the mean of the two tub experiments (October 15, 1986, and September 9, 1987) that examined particle selection down to 0.63 μm. This latter overall mean was the one we used to graphically portray *Lampsilis* W' versus ESD curve for natural seston (Fig. 4).

Plastic microspheres

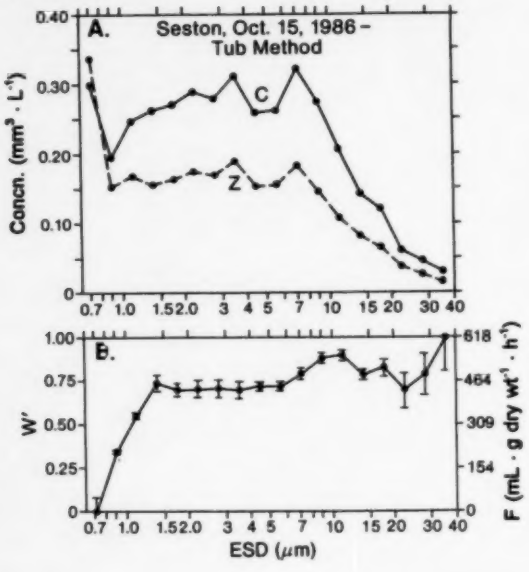
Results of the siphon experiments with plastic microspheres showed that microspheres were less efficiently retained than natural seston (Table 3). Unlike the curves for natural seston, W' versus ESD curves from the two experiments with the mixtures of 0.74 and 0.91 microspheres and *Chlamydomonas* did not have a smooth monotonic increase with increasing ESD (Fig. 5). We attribute the less than expected W' in the 1–3 μm ESD range to the extremely low concentration of particles in this region. Not only were there no or extremely few algae and microspheres in this region, there should have been little seston because the particles were suspended in 0.2-μm-filtered lake water. Possibly, the extremely sharp peaks in the concentration spectrum next to the low point could have biased results in this region because of coincidence counting (two small particles counted simultaneously to give a count for one larger particle) or peak smearing (particles being sized larger or smaller than true size) introducing false counts into this region. Possibly there was some particle production, although the siphon method should have minimized this. Whatever the cause, we considered it imprudent to draw any conclusions about particle selection in this region. The values reported in Table 3, therefore, include only the channels (size categories) of the microsphere and *Chlamydomonas* peaks.

The tub experiment with the mixture of 0.74-μm microspheres and *Chlamydomonas* sp. and *C. oblonga* (Fig. 6) and the siphon experiments with both sizes of microspheres (Fig. 5) showed that the 0.74-μm microspheres (0.63–0.79 μm channel) were poorly retained (Table 3) as might have been expected from the seston results (Table 2). However, the low W' value (0.07) for the 0.91-μm microspheres (Table 3) relative to the much higher value (0.44) for seston (Table 2; Fig. 4) was not expected.

Seasonal seston concentrations and filtration rates

Chlorophyll concentrations at the Lake St. Clair sampling site were relatively constant and low (Fig. 7; Table 4), indicative of oligotrophic–mesotrophic conditions (e.g., Vanderploeg 1990). This is not surprising because this site is strongly dominated by inflow of water from oligotrophic– mesotrophic Lake Huron (Leach 1972). Coulter-determined seston concentration generally paralleled chlorophyll concentration.

Fig. 2. *Lampsilis* in tubs filtering Lake St. Clair seston on October 15, 1986. (A) Mean concentration of particles as function of ESD in control (C) and experimental (Z) tubs at the end of the experiment. (B) Corresponding retention efficiency (W') and filtering rate (F) ± SE versus ESD curve that results from C and Z data. Since $W' = F/F_{pref}$, where F_{pref} is the filtering rate of the preferred size category (see Methods), one curve (with SE bars) with two different ordinate scales serves for both F and W'.



An extreme exception is the sample of November 1987, when the seston concentration was very high and the chlorophyll/seston ratio was very low (Fig. 7; Table 4). The high seston concentration was probably a result of resuspension of bottom sediments caused by the typically rough weather of November.

Seasonal filtration rate (F_{pref}), or pumping rate, exhibited a pattern that roughly paralleled the seasonal trend in temperature (Fig. 7). The relation between filtration rate and temperature was examined using the nonlinear regression relation $F_{pref} = Ae^{BT}$, where T = temperature (Table 5; Fig. 8). The regression gave a good fit in terms of both R^2 and size of SEs relative to coefficients (Table 5) (Kvalseth 1985). Although the regression appeared to follow the general trend of the data and had a high R^2 value, ANOVA using the pure error estimate from the replicate tubs (Fig. 8) showed highly significant ($P < 0.01$) lack of fit (Draper and Smith 1966); thus, not all seasonal variation in pumping rate was explained by temperature.

To explore the possibility that food concentration was an important determinant of filtering rate, we did nonlinear regressions of the relation $F_{pref} = A/(C + X)$, where X = "food" concentration expressed as Coulter-measured particle concentration (Fig. 9A) and as chlorophyll (Fig. 9B). This relation is the filtering rate relation derived from the Michaelis–Menton expression for ingestion (e.g., Vanderploeg et al. 1984). These regressions showed very poor fits to the

Fig. 3. Mean particle retention efficiency (W') ± SE as a function of ESD of five *L. r. siliquoidea* determined from siphon experiments with natural seston. *I* and *E*, respectively, denote mean particle concentrations going into the incurrent siphon and leaving the excurrent siphon.

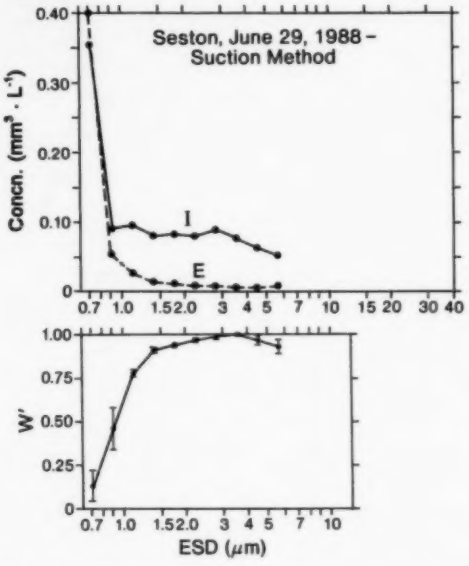
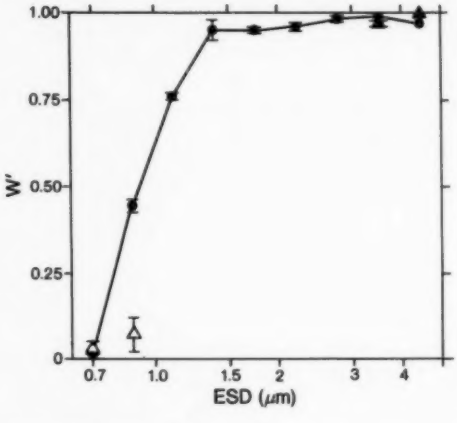


Fig. 4. W' (±SE) versus ESD curve for *L. r. siliquoidea* feeding in natural seston (solid circles) and results for mixtures of 0.74- and 0.91-μm microspheres (open triangles) with *Chlamydomonas* (solid triangles). The W' value of the 0.91- μm microspheres was significantly different ($P < 0.01$) from that of seston in the same size category (0.79–1.0 μm).



data (Table 5) both in terms of R^2 and large SEs on the coefficients.

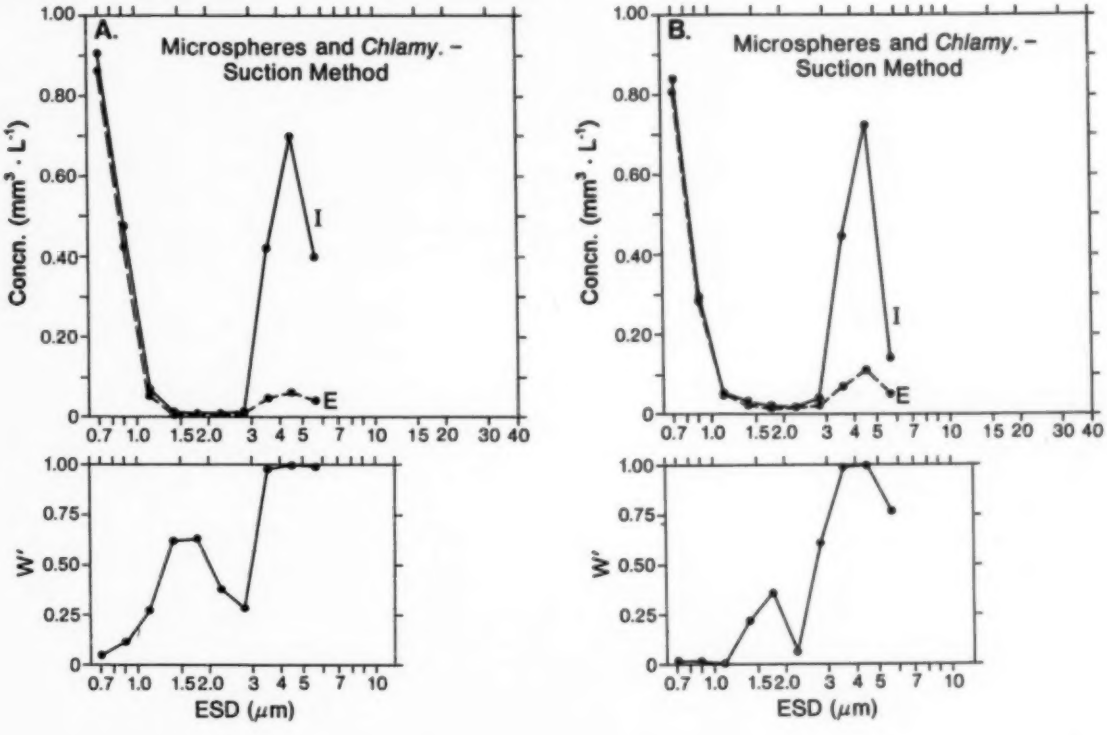
We also did a nonlinear regression for the product of the temperature relation and the concentration relation, i.e., $F_{pref} = Ae^{BT}/(C + X)$, where X = seston concentration. This combined-effects model (Table 5) did not result in a

Table 3. W' (±SE) of *L. r. siliquoidea* for 0.74- and 0.91-μm microspheres and *C. oblonga*.

Experiment	ESD (μm) 0.63–0.79	0.79–1.00	3.17–4.00	4.00–5.04
Tub				
Sept. 10, 1987[a]	0 ± 0.04	—	0.94 ± 0.19	1.00 ± 0.21
Siphon				
Sept. 24, 1987[b]	0.05 ± 0.01	0.12 ± 0.03	0.98 ± 0.24	1.00 ± 0.32
Nov. 24, 1987[c]	0.02 ± 0.03	0.0164 ± 0.03	0.99 ± 0.02	1.00 ± 0.02
Mean	0.03	0.07	0.97	1.00
SE	0.02	0.05	0.01	0.00

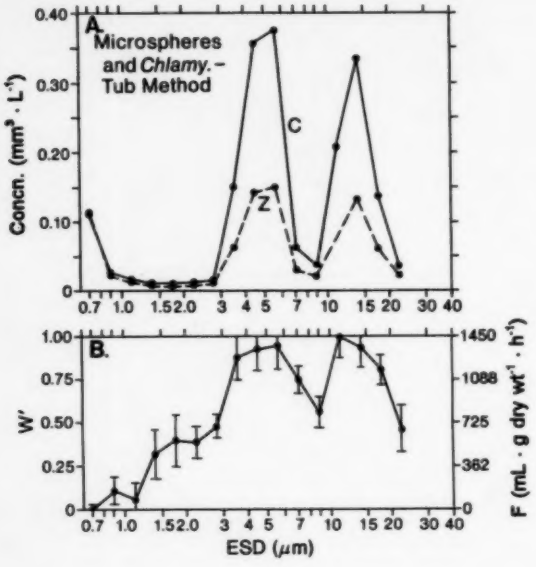
[a]Three mussels in each of four tubs; SE is for replicate tubs.
[b]One mussel; SE is projected (Bevington 1969) from counting error of replicate counts of a single large sample taken from the excurrent and the incurrent siphons.
[c]One mussel; SE is error calculated from seven separate samples from incurrent and excurrent siphons.

Fig. 5. W' versus ESD of *L. r. siliquoidea* determined from filtering efficiency in (A) the first (September 1987) and (B) second (November 1987) siphon experiments with a mixture of 0.74- and 0.91-μm microspheres and *C. oblonga*. The first two size categories (data points) correspond to the microspheres, and the last three size categories correspond to the biomass peak of *C. oblonga*. Abbreviations same as in Fig. 3.



larger R^2 than the model for temperature alone, and the fit was poor as demonstrated by the large SE on the coefficient C, the half-saturation coefficient on the Michaelis–Menton relation for the food concentration effect. Interestingly, the temperature effect, defined by the coefficient B, was approximately the same in the temperature and combined-effects models. Thus, the results in aggregate argue that temperature is the primary driving variable.

Fig. 6. *Lampsilis* in tubs filtering a mixture of 0.74-μm microspheres and *Chlamydomonas* sp. and *C. oblonga*. (A) Mean concentration of particles as a function of ESD in control (C) and experimental (Z) tubs at the end of the experiment. (B) Corresponding retention efficiency (W') and filtering rate (F) versus ESD curve that results from C and Z data.



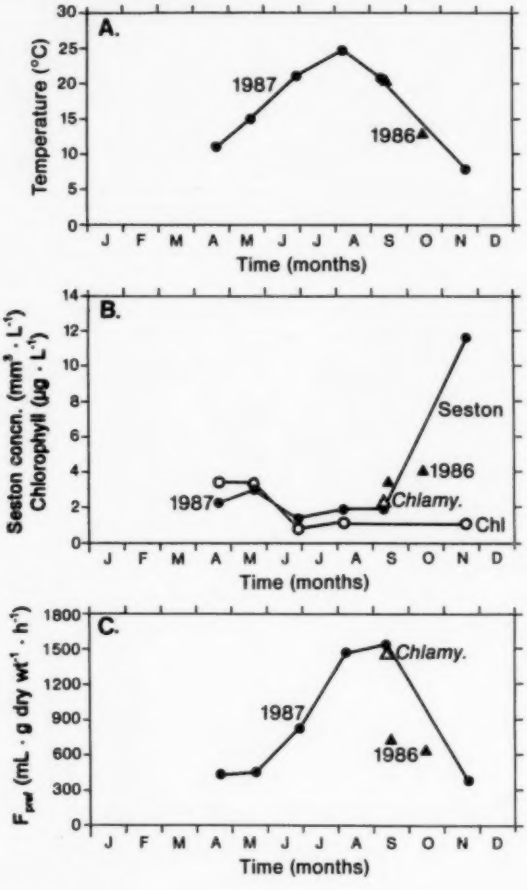
The experiments comparing filtering rates of the mussels buried in sediment and subsequently removed from sediment (Table 6) support the assumption that the tub method was giving a correct measure of filtering rate unbiased by possible effects of handling or need for burial in sediment that might lower the estimate of filtration rate (Kryger and Riisgard 1988; Reeders et al. 1989). Filtering rates on average for mussels lying on one valve were slightly but not significantly higher than their rates buried in sediment (Table 6). This is consistent with the observation that in both the buried and on-side positions, the valves and siphons appeared to be fully open, an indication of maximum pumping rate (Jorgensen 1990).

Discussion

Particle-retention efficiency

Lampsilis had significant retention efficiencies for natural seston <1 μm ESD with zero efficiency reached for particles <0.7 μm ESD. Our result is the first to document retention efficiencies for seston <1 μm by any bivalve. This means that *Lampsilis* can filter nearly all sizes of algae including small picoplankton. Significant retention efficiencies for particles less than 1 μm are also expected in other bivalves because many marine species have W' values of 0.40–0.70 for seston of 1.1 μm ESD (Mohlenberg and Riisgard 1978; Jorgensen et al. 1984). Because the mechanism of bivalve filtering is not understood, we can only speculate as to why submicrometre microspheres were not retained as efficiently as seston of the same ESD. The

Fig. 7. Seasonal patterns of (A) temperature, (B) food concentration (expressed as Coulter-measured seston concentration and chlorophyll), and (C) pumping rate (F_{pref}) of *L. r. siliquoidea* in Lake St. Clair. Solid triangles and solid circles refer, respectively, to 1986 and 1987 data. For a comparison of filtering on natural seston and laboratory algae (plus beads), results for the same mussels feeding on cultured algae the next day at the same temperature (Fig. 6) are also plotted (open triangles) in Fig. 7B (seston concentration) and 7C (F_{pref}).



bivalve filter does not act as a mechanical sieve, but complex laminar currents at the entrance to interfilament canals (pores) somehow transfer particles across the streamlines of water flow through the pores (Jorgensen 1990). Perhaps shape of the seston plays a role in this transfer. Seston, whether clay, organic detritus, algae, or bacteria, is often elongated in one or two dimensions. Perhaps an elongated particle of seston of 0.9 μm ESD is more likely to be transferred across the streamlines (and not through the pores) than a sphere of the same ESD.

Because we do not know the energetic costs of filtration as it relates to particle size retention efficiency in bivalves, we cannot evaluate the advantages of retaining picoplankton. However, we can say picoplankton and other particles

Table 4. Mean pumping rates (±SE, $N = 4$), F_{pref}, of *L. r. siliquoidea* as a function of environmental variables.

Date	Temp. (°C)	Chl[a] ($\mu g \cdot L^{-1}$)	Available particles: ESD range (μm)	Available particles: Concn.[a] ($mm^3 \cdot L^{-1}$)	Chl/particles ratio ($\mu g \cdot mm^{-3}$)	F_{pref} ($mL \cdot g$ dry $wt^{-1} \cdot h^{-1}$)
Sept. 15, 1986	20.0	—	1–40	3.39[b]	—	721 ± 19
Oct. 15, 1986	13.0	—	1–81	3.99	—	618 ± 115
Apr. 21, 1987	11.0	3.42	1–81	2.32[b]	1.47	420 ± 67
May 20, 1987	15.0	3.30	1–81	2.97	1.11	452 ± 62
June 30, 1987	21.0	0.82	1–40	1.27[b]	0.64	842 ± 56
Aug. 5, 1987	24.6	1.10	1–81	1.92	0.57	1454 ± 63
Sept. 9, 1987	21.5	1.89	1–40	1.92	0.99	1532 ± 146
Nov. 19, 1987	8.0	3.14	1–40	11.70	0.27	503 ± 79[c]

[a] SE < (0.03) $\bar{X}$.

[b] Particle concentration was measured down to 2 μm and extrapolated down to 1 μm by assuming that concentration in two missing channels was equal to that of the two adjacent channels.

[c] One of the mussels (replicates) did not open its siphon; therefore, this mussel (replicate) was excluded from analysis.

Table 5. Summary statistics for nonlinear least squares fits of pumping rate, F_{pref} ($mL \cdot g$ dry $wt^{-1} \cdot h^{-1}$) of *L. r. siliquoidea* on Lake St. Clair seston to relations for temperature (T, °C), food concentration (X) expressed as Coulter-measured seston concentration ($mm^3 \cdot L^{-1}$) and chlorophyll concentration ($\mu g \cdot L^{-1}$), and combined effects of temperature and food concentration. Asterisks indicate values not significantly different from zero at the 5% level. ANOVA using pure error analysis of replicate tubs showed significant lack of fit at the 1% level for all regressions.

Effect/relation	R^2	Coefficients (±SE): A	B	C	Correlation between: A and B	A and C	B and C
Temperature							
$F_{pref} = Ae^{BT}$	0.92	161 ± 51	0.090 ± 0.015		−0.98		
Concentration							
$F_{pref} = A/(C+X)$							
where X = seston	0.82	4301 ± 2079		2.41 ± 2.17*		0.98	
where X = Chl	0.84	3774 ± 2606*		2.15 ± 2.77*		0.99	
Combined effects							
$F_{pref} = Ae^{BT}(C + X)$							
where X = seston	0.92	−3624 ± 4143*	0.099 ± 0.018	−29.3 ± 22.9*	0.81	0.96	0.64

in this size range represent a significant resource. Fahnenstiel and Carrick (1992) showed that picoplankton accounted for 10% of the phototropic biomass and 40% of phototrophic production in Lakes Michigan and Huron. It is noteworthy that small phototrophic plankton, such as *Synechococcus*, that passed through a 1-μm screen accounted for 17% of the phototrophic production in their study. Their study did not document the contribution of bacteria or detritus that may also be available to the mussels.

The contribution of particles <3.17 μm ESD to the total particle size spectrum in Lake St. Clair was quite large, ranging between 42 and 49% (Fig. 10; Table 7). If we restrict our consideration to particles in the 1.00–3.14 μm size range, which mussels can retain quite efficiently, the contribution was 28–39%. Thus, it seems likely that particles <3 μm ESD are a potentially important food resource to the mussels. Of course, some of the particulate material in all size categories is abiotic seston; therefore, we cannot specify precisely what portion of mussels' food was smaller than 3 μm ESD.

Filtering rate

The range of weight-specific filtering rates in our study was within the range of values measured by Kryger and Riisgard (1988) using the siphon technique for various species of unionids feeding on laboratory algae (Table 8). Particularly good agreement was noted when our results for *Lampsilis* at 20–22°C were compared with results for *Anodonta* of the same temperature regardless of whether seston or laboratory algae was the food source. Confidence in our results is also implied by the close agreement between filtering rates of buried mussels and mussels lying on one valve (Table 6).

Kryger and Riisgard (1988) noted that their values were at least four times higher than in other studies and attributed this difference to poor food quality of experimental

Table 6. Comparison of filtering rates (F_{pref}) on *C. oblonga* by *L. r. siliquoidea* (2.1–2.7 g dry wt) buried in sand or lying on their sides (on one valve). F_s/F_B is the ratio of F_{pref} values for mussels on their sides (S) to those buried (B) in sand. Algal concentrations are geometric means of algal concentrations at beginning and end of feeding periods. A paired *t*-test indicated that differences in F_{pref} between experimental treatments were not significantly different ($P > 0.1$).

		Buried		On side		
Mussel	Date	Algal concn. ($mm^3 \cdot L^{-1}$)	F_{pref} ($mL \cdot g$ dry $wt^{-1} \cdot h^{-1}$)	Algal concn. ($mm^3 \cdot L^{-1}$)	F_{pref} ($mL \cdot g$ dry $wt^{-1} \cdot h^{-1}$)	F_s/F_B
A	Sept. 4	0.52	2317	0.70	2496	1.08
	Sept. 9	0.55	1648	0.45	1726	1.05
B	Sept. 4	1.04	881	0.89	1426	1.62
	Sept. 9	0.82	1211	1.13	907	0.75
	Sept. 10	0.62	896	0.60	1344	1.50
C	Sept. 4	1.01	1413	0.72	1123	0.79
	Sept. 10	0.72	1142	0.52	1110	0.97
D	Sept. 4	0.95	579	1.01	763	1.32
	Sept. 10	0.71	779	0.78	1121	1.44
E	Sept. 10	1.41	663	1.18	929	1.40
$\bar{X}$		0.84	1153	0.80	1294	1.19
SE		0.09	167	0.08	160	0.10

Fig. 8. Nonlinear regression of pumping rate (F_{pref}) of *L. r. siliquoidea* in Lake St. Clair to the relation $F_{pref} = Ae^{BT}$, where T is temperature. The arrow with the number 2 points to two values so close that they cannot be distinguished from each other.

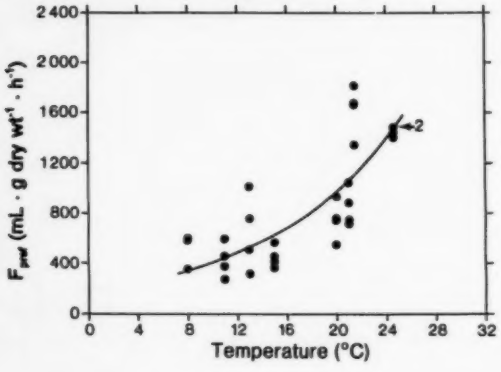
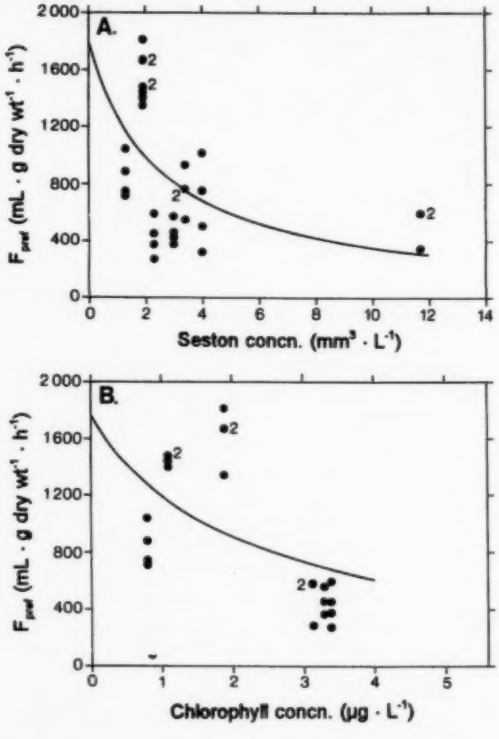


Fig. 9. Nonlinear regression of F_{pref} (pumping rate) of *L. r. siliquoidea* to the relation $F_{pref} = A/(C + X)$, where X = food concentration expressed as (A) Coulter-measured seston concentration and (B) chlorophyll concentration.



particles, high food concentration, and experimental methods (whether buried in sediment or otherwise disturbed) of these other studies. Our finding that filtration rates of mussels were similar in and out of sediment suggests that food concentration and quality were likely the main factors contributing to previous discrepancies.

The clearance method is simple in design and easy to set up compared with the siphon method. However, not all mussels may filter well like *Lampsilis* in the absence of sediment; therefore, before using the clearance method for other species, it would be prudent to check to see that they filter at the same rate in and out of sediment. Also, other species like *D. polymorpha* may have reduced filtering rates for several hours after disturbance (Reeders et al. 1989). Both these potential problems could probably be evaluated by observing the degree to which the valves and siphons open (Jorgensen 1990).

Table 7. Percent contribution of particles in the ESD size range of picoplankton (ESD < 3 μm) to total volume of seston measured by the Coulter counter in those experiments measuring particles ≤1 μm. The 1.0–3.17 μm size category, which is efficiently filtered by *Lampsilis*, is distinguished from the broader size range of 0.63–3.17 μm.

	ESD size range (μm)	
Date	0.63–3.17	1.00–3.17
Oct. 1986	42.5	35.3
May 1987	34.9	28.4
Aug. 1987	47.4[a]	39.6
Sept. 1987	49.1[a]	38.7

[a]Particle concentration was measured down to 1 μm and extrapolated down to 0.63 μm by assuming that concentration in two missing channels was the same as in the two adjacent channels.

Fig. 10. Importance of particles in the picoplankton size range to particle size spectra of Lake St. Clair seston. The shaded area (particles <3 μm ESD) shows the portion of the spectrum in the picoplankton size range. The curves are the mean initial control concentrations from the tub experiments with *Lampsilis* that measured particles ≤1 μm ESD.

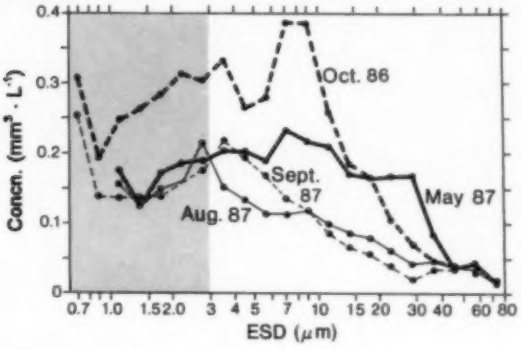


Table 8. Comparison of filtration rates of *L. r. siliquoidea* (this study) with those of other freshwater unionids in the study of Kryger and Riisgard (1988).

Species	Particles	Method	Dry wt (g)	Temp. (°C)	Filtering rate (mL·g dry wt^{-1}·h^{-1})[a]
L. r. siliquoidea	Seston	Clearance	1.5–2.1	8–25	382–1532
L. r. siliquoidea	Seston	Clearance	1.5–2.1	20–21	721–1532
L. r. siliquoidea	*Chamydomonas* spp.	Clearance	1.5–2.1	22	1450
Anodonta anatina	*Chlorella vulgaris*	Siphon	1.5–2.1	19–20	944–1006[b]
Unio pictorum	*C. vulgaris*	Siphon	3.0	19–20	400–567
Unio tumidus	*C. vulgaris*	Siphon	2.4	19–20	542–625
Unio crassus	*C. vulgaris*	Siphon	2.7	19–20	444–519

[a]F_{pref} is used as measure of filtering rate for *L. r. siliquoidea* data.
[b]Filtration rate range predicted from regression analysis for weight range shown.

The pumping rates obtained for *Lampsilis* feeding on natural seston are consistent with Jorgensen's (1990) assertion that mussels, except under conditions of stress such as low food or oxygen concentrations, pump with siphons fully open at maximum rate that increases with temperature. First, seasonal variation in temperature accounted for most of the variation in pumping rate. Some of the unexplained variation in the pumping rate (expressed per unit weight) versus temperature regression for *Lampsilis* relates to inevitable seasonal variation in gonad mass. Second, rates determined for *Lampsilis* on natural seston were comparable with those for European unionids (Kryger and Riisgard 1988) at the same temperatures in siphon method experiments with cultured algae. Our data are particularly valuable because, by using mussels freshly collected from the field during different seasons, we were using animals fully acclimated to temperature and other environmental variables.

Our filtration rate data were used to determine the significance of unionid filtering by the unionid community in Lake St. Clair. *Lampsilis* was the dominant unionid in the lake (Nalepa and Gauvin 1988), and we assumed that all

Table 9. Filtration of *L. r. siliquoidea* and the total mussel community expressed as the fraction of the total lake volume cleared per day.[a]

	Community filtration (d^{-1})	
Date	*Lampsilis*	Total
Sept. 15, 1986	0.009	0.024
Oct. 15, 1986	0.008	0.021
Apr. 21, 1987	0.005	0.014
May 20, 1987	0.006	0.016
June 30, 1987	0.010	0.029
Aug. 5, 1987	0.017	0.050
Sept. 9, 1987	0.018	0.053
Nov. 19, 1987	0.006	0.017

[a]Calculated as (F_{pref} of *Lampsilis*) × (mussel mass per unit area) ÷ (mean depth of lake). Masses of *Lampsilis* and total mussels were, respectively, 1.54 and 4.40 g·m^{-2}; mean depth of lake = 3.06 m (Nalepa and Gauvin 1988).

unionids had the same weight-specific filtration rate as that measured for *Lampsilis*. In view of the excellent agreement between filtering rate of *Lampsilis* and European bivalves, this appears to be a reasonable assumption. The portion of the total lake volume cleared per day varied seasonally between 1.4 and 5.3% (Table 9).

Nalepa (1994) is monitoring the replacement of native unionids by the invading *D. polymorpha* in Lake St. Clair. Our size-selective filtration for native unionids combined with information on *Dreissena* filtering rates will provide a basis for evaluating changes in community filtering and, thus, ecological impacts.

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Gillnet catches as an estimate of fish abundance: a comparison between vertical gillnet catches and hydroacoustic abundances of Baltic Sea herring (*Clupea harengus*) and sprat (*Sprattus sprattus*)

Sture Hansson and Lars G. Rudstam

Abstract: We compared the catches in a series of six vertical gill nets (6.25- to 18.75-mm bar mesh) with estimates of fish abundance derived from hydroacoustics. A total of 47 combined acoustic and gillnet surveys were collected between July and November 1987–1992 in archipelago areas of the northern Baltic Sea. Fish abundance determined by hydroacoustics was significantly correlated with gillnet catches but explained only 28% of the variance in catch per unit effort corrected for gillnet selectivity and fish swimming speed. Visibility (measured as Secchi disk depth) significantly decreased the likelihood of fish being caught in gill nets, while the effect of temperature was positive but nonsignificant. These data imply that gillnet catches will be a biased indicator of changes in fish abundance over time or space, if visibility and possibly also temperature change as well.

Résumé : Nous avons comparé les captures réalisées avec une série de six filets maillants verticaux (maillage de 6,25 à 18,75 mm) aux estimations de l'abondance des poissons obtenues par des moyens hydroacoustiques. Au total, 47 relevés combinant les moyens acoustiques et les filets maillants ont été effectués entre juin et novembre, de 1987 à 1992, dans certaines zones de l'archipel du nord de la Baltique. L'abondance des poissons mesurée par des moyens hydroacoustiques présentait une corrélation significative avec les captures réalisées aux filets maillants mais expliquait seulement 28 % de la variance des prises par unité d'effort après correction en fonction de la sélectivité des filets maillants et de la vitesse de nage des poissons. La visibilité (mesurée au disque de Secchi) réduisait de façon significative la probabilité de capture des poissons dans les filets maillants, tandis que l'effet de la température était positif mais non significatif. Ces données semblent indiquer que les prises réalisées aux filets maillants constituent un indicateur biaisé des changements de l'abondance des poissons dans le temps ou dans l'espace si la visibilité, et peut-être la température, sont modifiées.

[Traduit par la Rédaction]

Introduction

Gill nets are used extensively by fisheries managers for population assessment (e.g., Hamley 1975; Hubert 1983; Hansson 1987; Degerman et al. 1988). To be useful, net catches have to provide at least a relative index of fish abundance. Many authors have derived methods for estimating the size selectivity curves of different mesh sizes (review by Hamley 1975; recent examples in Wulff 1986; Hesler et al. 1990; Henderson and Wong 1991). These curves can be constructed from catches in a range of mesh sizes. But gill nets are passive fishing gear that the fish has to encounter to be captured. Hence, catches depend on fish activity (Hamley 1975; Rudstam et al. 1984). Fish activity, in turn, may depend on light, temperature, abundance of prey, predators, other interfering species, hunger levels, physiological condition (i.e., spawning), and other factors (Neuman 1979; Rudstam et al. 1984; Nyberg and Degermán 1988; Borgstrøm 1992). Activity will therefore vary with fish size and season. Net catches are also affected by the visibility of the mesh and consequently by light and turbidity (Berst 1961). In contrast with mesh selectivity curves, these effects of fish activity and mesh visibility cannot be evaluated indirectly from the net catches alone. It is necessary to evaluate these factors directly, by sampling

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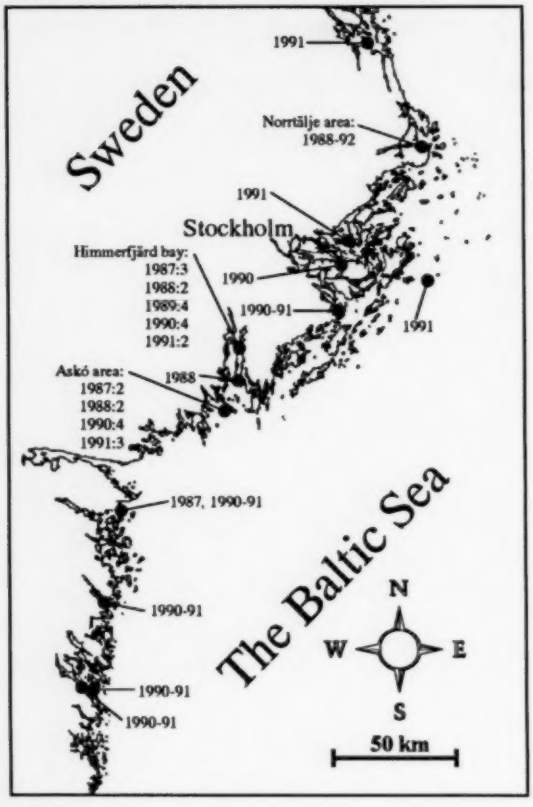
Fig. 1. Study area on the Swedish east coast. Black circles show survey areas. If an area was fished only once in a year, that year or range of years is given next to the circle. In Himmerfjärd Bay and the Askö area, repeated studies were made each year, the number of which is given next to the year.



a known fish population. Such comparisons have only been made for a few species (walleye (*Stizostedion vitreum*), Hamley and Regier 1973; roach (*Rutilus rutilus*), Borgstrøm 1989; brown trout (*Salmo trutta*), Borgstrøm 1992).

In archipelago areas of the Baltic Sea, we have used vertical gill nets combined with hydroacoustics since 1985. Our approach has been to use acoustics to measure abundance and to use catches in gill nets to estimate species composition and to obtain samples for diet analyses (Rudstam et al. 1988, 1992; Hansson and Rudstam 1990; Hansson 1993). Generally, 95% or more of the pelagic fish we caught were clupeids, herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). In this paper, we present mesh selectivity curves for clupeids calculated from the catch data, and we compare corrected and uncorrected gillnet catches with acoustic estimates of abundance. Then, we derive a model for including visibility and temperature effects on the efficiency of gillnets to capture fish and use this model to test for effects of visibility and temperature on the relationship between gillnet catches and acoustic abundance.

Materials and methods

A total of 47 combined acoustic and gillnet surveys have been conducted in the Swedish archipelago areas of the northern Baltic (from 57°48′N to 60°11′N; Fig. 1). The surveys were done between July and November (1987–1992) which is after the spawning period and during the feeding season for herring in this part of the Baltic. For 43 of the 47 surveys, we also measured temperature (average 12.7°C, range 6.5–19.6°C) and water transparency (Secchi disk depth, average 4.9 m, range 9.1–2.0 m). Most of the areas included in this study were surveyed more than once, and in these cases, gillnet stations and hydroacoustic transects were the same each time.

Hydroacoustics

Hydroacoustic data were collected at night, when these fish populations are dispersed. Generally we used small boats (length <10 m) operated at speeds of 4–7 kn, with a 70-kHz single-beam echo-sounder (Simrad EY/M, half power beam width 5.6°, sound pulse length 0.6 ms, 3 pulses (pings) per second, TVG of 40 log R). The transducer depth was 1 m and the echo analysis started 2 m from the transducer (i.e., 3 m below the surface). The unit was calibrated each year with a −39.2-dB standard copper sphere (MacLennan and Simmonds 1992) and differences between years were small (less than ±1 dB). The 10-kHz output signal was recorded on cassette tapes and analysed using the program HADAS version 3.3 (Lindem 1983; Rudstam et al. 1988; Walline et al. 1992). This program uses a modification of the Craig and Forbes (1969) algorithm to remove the beam pattern effect. Only nonnegative solutions to the system of linear equations are accepted and negative values are set to 0. Echo integration, fish density, and target strength distribution are determined for single-fish echoes (echoes with a length of less than twice the pulse length at the noise threshold) for each survey. Echo integration values from multiple-fish echoes (echoes longer than twice the pulse length) are converted to fish abundance using the relationship between fish density and echo integration determined from the single-fish echoes from the same survey. This analysis of single-beam data gives abundance and target strength distributions similar to the dual-beam method (Foote 1991; Dahm et al. 1992; Parkinson et al. 1994). Acoustic abundances were based on targets larger than −56 dB, corresponding to clupeids larger than approximately 5 cm (using the relationship between target strength and fish length derived by Rudstam et al. (1988) for Baltic herring and sprat at 70 kHz, $TS = 20 \log L - 69.9$).

Analyses were performed in 2-m depth intervals and summed over the whole water column to give a total abundance. For the present analysis, we included sections of acoustic surveys that are within 1 nautical mile of the gillnet sampling station. In most cases, this translated to a single linear transect with the gillnet station located in the middle of the transect. A transect length of 2 nautical miles is a compromise between the need to analyse a sufficient number of pings for accurate fish density estimates and the need for local fish density estimates around the nets. Our experience is that 2000–4000 pings are needed to get reliable acoustic abundance estimates (Hansson 1993), and

2 nautical miles represent about 4000 pings. Furthermore, when gill nets are used in fish surveys, they are assumed to be representative of a certain area, not only the point where they are located. In coastal areas and large lakes, a pelagic area with a radius of 1 nautical mile is considered small. In addition to this, fish move during the period the nets are set. During 8 h (common net set), herring are likely to swim 1 nautical mile or more (Boyar 1961; Blaxter and Hunter 1982).

Gill nets

The vertical gill nets used, described in detail by Hansson (1988), were either 3 m wide and 30 m deep (1987–1990) or 1.5 m wide and 25 m deep (1990–1992). The nets were made of monofilament nylon yarn with one mesh size per net. Catches in the 1.5-m-wide gill nets were doubled to make them comparable with those in the 3-m-wide nets (for justification, see Discussion). At each fishing station, we used six gill nets (bar mesh sizes 6.25, 8, 10, 12, 15, and 18.75 mm). The nets were set overnight at either 28–30 or 23–25 m depth, covering the water column from the bottom to the surface. Fish were identified to species and counted in the field. All fish were measured (total length) in the laboratory.

Catches (Catch) in a fishing gear are related to the density (N) of the population of interest by a catchability coefficient q, defined by the equation

(1) $q = \text{Catch}/N.$

For passive fishing gears such as gill nets, the catchability coefficient is the product of two probabilities: the probability that a fish encounters the net (P_e) and the probability that the fish is caught and retained after encountering the net (P_r) (Hamley 1975; Rudstam et al. 1984; Borgstrøm and Plahte 1992):

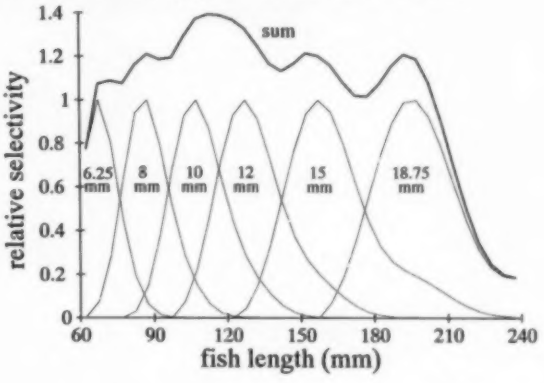
(2) $q = P_e \cdot P_r.$

Because both P_e and P_r may vary with fish length, q is a function of fish length. For gill nets, P_r is strongly dependent on fish size; a gill net of a given mesh size catches a relatively narrow size range. Several equations have been used to describe the selectivity curves (including normal, skew-normal, and gamma functions) derived from the size distributions of a catch (see review by Hamley 1975). These selectivity curves are then used to remove the bias associated with the mesh size itself. We used the skew-normal functions (Wulff 1986)

$$(3)\quad S(L,m) = \exp\{-1/2\cdot\lambda^2\}\cdot\{1 - k/2\cdot\sigma(m)^{3/2}\cdot[\lambda - 1/3\cdot\lambda^3]\}$$
$$\lambda = (L - L_0(m))/\sigma(m)$$
$$L_0(m) = a\cdot m$$
$$\sigma(m) = b\cdot m$$

where $S(L,m)$ is the selectivity for a fish of length L (millimetres) in a net with mesh size m (bar mesh, millimetres), $L_0(m)$ is the modal fish length in mesh size m, $\sigma(m)$ is the standard deviation for mesh m, and k is the skewness constant. We estimated the parameters a, b, and k using gillnet catches of herring on seven sampling occasions during 1987 and the maximum likelihood estimator technique described by Wulff (1986). The resulting values for these parameters are $a = 11$, $b = 1.2$, and $k = 0.016$. In eq. (3), L_0 and σ are proportional to m. Adding an intercept to these relationships (Wulff 1986) only marginally improved the fit. This equation can yield negative values for L far from L_0. Such values were set to 0. Finally, we assume that all nets have the same retention probability for the most efficiently caught fish size (the modal length). Therefore, we standardized the selectivity functions for each mesh size by dividing with the maximum value of S for that mesh size. The relationship between modal length of a net and mesh size in these curves is very similar to curves constructed using girth-to-length relationships from catches in this area in 1985, using a smaller subset of these nets (Rudstam et al. 1988). The curves are unimodal and skewed to the right. Because herring may be caught wedged, gilled, and tangled in gill nets, the mesh selectivity curves may be multimodal (Winters and Wheeler 1990). There was no indication of any multimodal curves in our catch data.

Fig. 2. Selectivity curves for each mesh size and the combined selectivity profile for the set of six nets.



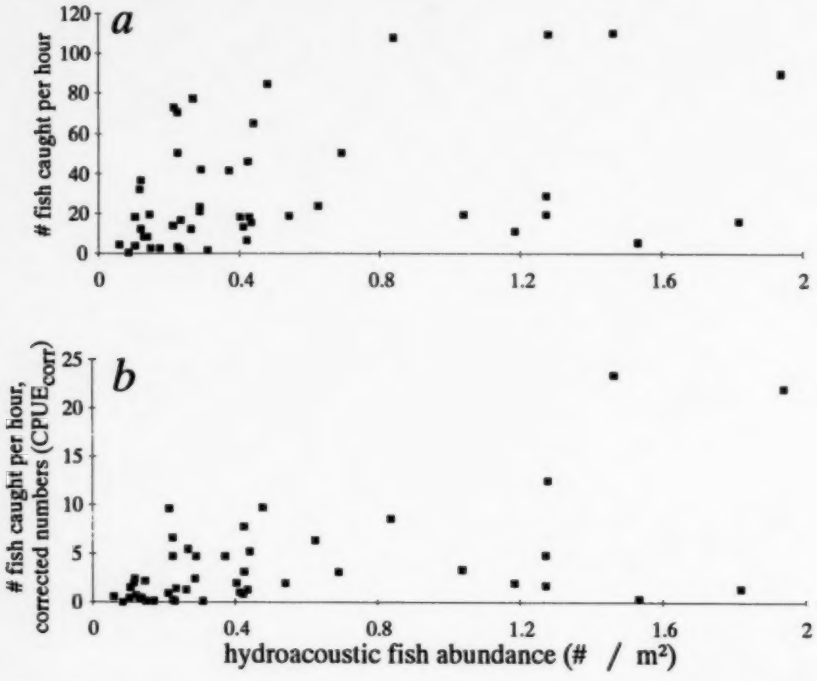
By using a gang of gill nets with overlapping selectivity curves, and by correcting the total catch with the total selectivity of all mesh sizes for fish size ($S(L)$), we minimize errors associated with catches of a few fish far from the modal length of a net (Jensen 1986). For fish of a given size (L), we now have the following equation for P_r:

$$(4)\quad P_{rL} = a_1 \cdot \sum_m S(L,m) = a_1 \cdot S(L).$$

In this and the following equations, a_x are constants. The resulting total selectivity function is fairly even for fish sizes between 6 and 24 cm total length (Fig. 2). Herring seldom reach sizes larger than 24 cm in the northern Baltic Sea proper, and sprat never reach this size (Hagström et al. 1991). We use the $S(L)$ curve to correct gillnet catches of all clupeids, including sprat. Sprat have a length-to-girth relationship similar to herring (Rudstam et al. 1988), suggesting similar net selectivity curves. The size distribution of the sprat caught were too small to construct reliable net selectivity curves specifically for sprat.

Rudstam et al. (1984) suggested that encounter probability is proportional to swimming speed and that swimming speed is a function of fish size. Rudstam et al. (1984) found that volitional swimming speed of bloater (*Coregonus hoyi*) in a large aquarium was proportional to fish length to the 0.8 power. Henderson and Wong (1991)

Fig. 3. Catches in gill nets compared with hydroacoustic fish abundance. (*a*) Catches expressed as number of fish caught in six vertical gill nets per hour, (*b*) identical to Fig. 3*a*, but corrected for gillnet selectivity and gillnet encounter probability ($CPUE_{corr}$ in eq. (6)).



assumed that swimming speed of walleye was proportional to fish length to the 0.5 power. Following Boyar (1961) and Blaxter and Hunter (1982), we assume that clupeid swimming speed is directly proportional to fish body length. Exponents lower than 1 in the relationship between fish length and swimming speed gave poorer relationships between acoustic fish abundances and gillnet catches. The expression for P_e for fish of length L is then

$$(5) \quad P_{e,L} = a_2 \cdot L.$$

The sum of the catches in all nets per hour is the catch per unit effort (CPUE). To adjust catches for the selectivity of the gill nets ($S(L)$, eq. (4)), and for length-dependent differences in swimming speed (proportional to L, eq. (5)), we calculated a corrected catch per unit effort ($CPUE_{corr}$). This was done by first dividing CPUE into catches per 0.5-cm-wide length groups ($CPUE_{Li}$, where L_i is the midpoint of length class i). From these, $CPUE_{corr}$ was estimated as

$$(6) \quad CPUE_{corr} = \sum_i CPUE_{L_i}/[L_i \cdot S(L_i)].$$

To estimate effects of gillnet mesh selectivity alone ($CPUE_{mesh}$), the factor L_i is removed from eq. (6).

The effect of fish length on both encounter and retention probability is accounted for in $CPUE_{corr}$. With a fish abundance N, we now define the residual catchability Q as

$$(7) \quad Q = CPUE_{corr}/N.$$

This residual catchability is a complex function of factors not included in eq. (4) and (5), but influencing the ability of gill nets to capture fish. We hypothesize that both temperature and transparency of the water are such additional factors. It is likely that swimming speed and hence the encounter probability is a function of temperature. We will test for this by assuming that the effects of fish length and temperature on swimming speed are indepedent and that the effect of temperature on catchability can be described by a linear function (proportional to $(a_3 + a_4 \cdot \text{Temp})$). Further, it is reasonable to assume that the number of fish caught will increase if a fish has difficulty detecting a net, and therefore, catchability should be a negative function of water transparency. Here, we assume that this effect is independent of fish size and a linear function of Secchi disk depth (proportional to $(a_5 + a_6 \cdot \text{Secchi})$). With these assumptions, we can write

$$(8) \quad Q = a_7(a_3 + a_4 \cdot \text{Temp})\,(a_5 + a_6 \cdot \text{Secchi})$$

which rearranged and inserted into eq. (7) is

$$(9) \quad CPUE_{corr}/N = a_8 + a_9 \cdot \text{Temp} + a_{10} \cdot \text{Secchi} + a_{11} \cdot \text{Secchi} \cdot \text{Temp}.$$

Based on these assumptions, we tested for effects of temperature and Secchi disk depth on residual gillnet catchability by fitting our data to eq. (9). As an estimate of N, the hydroacoustic fish abundance (N_{sonar}) was used. We tested for effects of temperature alone, Secchi disk depth alone, and for the complete model.

Table 1. Results of regression analyses, with two different expressions of catch per unit effort or catchability as dependent variables and hydroacoustic fish abundance, temperature, and Secchi disk depth as independent variables.

Regression model				Parameter estimates			
Dependent variable	Independent variables	r^2	p-value	Variable	Coefficient	t-value	p-value
(a) CPUE: catch per hour	Abundance	0.13	<0.02	Intercept Abondance	19.7 22.9	3.17 2.60	<0.01 <0.02
(b) $CPUE_{mesh}$: catch per hour with correction for mesh selectivity but not for gillnet encounter probability (L_i = 1 in eq. (6))	Abundance	0.23	<0.001	Intercept Abundance	14.3 33.3	2.26 3.72	<0.03 <0.001
(c) $CPUE_{corr}$: catch per hour with correction for mesh selectivity and gillnet encounter probability (eq. (6))	Abundance	0.28	<0.001	Intercept Abundance	1.05 5.37	1.16 4.20	0.25 <0.001
(d) $CPUE_{corr}/N_{sonar}$: residual catchability Q, estimated as catch per hour corrected for mesh selectivity and gillnet encounter probability (eq. (6)) divided by hydroacoustic fish abundance	Temperature	0.06	0.11	Intercept Temperature	0.73 0.67	2.05 2.23	0.88 0.11
(e) $CPUE_{corr}/N_{sonar}$: residual catchability Q, estimated as described for model d above	Secchi depth	0.10	<0.05	Intercept Secchi depth	15.9 −1.40	4.45 −2.09	<0.001 <0.05
(f) $CPUE_{corr}/N_{sonar}$: residual catchability Q, estimated as described for model d above	Temperature Secchi depth Temp·Secchi	0.11	0.22	Intercept Temperature Secchi depth Temp·Secchi	7.61 0.57 −0.43 −0.061	0.48 0.50 −0.16 −0.28	0.63 0.62 0.87 0.78
(g) $CPUE_{corr}$: catch per hour corrected for mesh selectivity and gillnet encounter probability (eq. (6))	Abundance·Q (Q = residual catchability, estimated from model e above)	0.38	<0.001	Intercept Abundance·Q	0.97 0.57	1.15 0.11	0.26 <0.001

Statistics

Linear regression (SAS 1987*a*, pp. 774–875) was used to analyse the effects on the dependent variable (gillnet catches and catchability coefficients) by the independent variables (fish abundance (number per square metre), water temperature at 10 m depth (degrees Celsius), and Secchi disk depth (metres)). We used the water temperature at 10 m because this is above the thermocline when the water is thermally stratified. Most herring are found above the thermocline at night during this period (Rudstam 1988; S. Hansson and L.G. Rudstam, unpublished observations). During mixed conditions, the temperature at 10 m is representative of the entire water column.

Results

There was a weak, but statistically significant positive relationship between vertical gillnet catches and hydroacoustic fish abundance (Fig. 3*a*; regression model *a* in Table 1). The correlation improved if catches were corrected for gillnet selectivity ($CPUE_{mesh}$; regression *b* in Table 1), but the resulting model has a significant positive intercept, predicting catches even in the absence of fish. This intercept disappears and the correlation improves if the gillnet encounter probability is also accounted for ($CPUE_{corr}$; regression *c* in Table 1; Fig. 3*b*). Effects of gillnet saturation are not likely the explanation of the overall

Table 2. Results from linear regressions between catches ($CPUE_{corr}$) and hydroacoustic fish abundance on subsets of data. Each of these subsets was collected at the same time of the year, either at different stations the same year or different years but at the same station. Locations of stations are shown in Fig. 1. None of the subsets produced statistically significant regressions. To test if data in these subsets are more strongly correlated than the data in the full data set (47 observations), random samples with either 4, 5, 8, 9, 10, or 17 observations were taken from the full data set (=bootstrapping). For each of the six sample sizes, 200 samples were taken and for each of these a linear regression analysis was performed. From the resulting 200 r^2 values, the five lowest and the five highest were removed and the interval of remaining values was used as an estimate of the 95% confidence interval (CI) of r^2 values generated from randomly drawn observations from the full data set. As can be seen from the table, the r^2 values of data subsets A–J are all within the corresponding confidence intervals. There was thus no marked tendency for the correlation to improve when only samples from the same area and/or the same time of the year were considered.

Data subset	Number of observations	r^2 value	95% CI of r^2
(A) Coastal stations fished Oct. 23 – Nov. 15, 1990	8	0.06	0.001–0.92
(B) Coastal stations fished Oct. 1–15, 1991	9	0.05	0.001–0.92
(C) Coastal stations 1990–91 (data set A + B)	17	0.01	0.002–0.79
(D) Askö area, July 13 – Aug. 1, 1987–1991	4	0.24	0.001–0.99
(E) Askö area, Aug. 15 – Sept. 8, 1987–1991	4	0.06	0.001–0.99
(F) Askö area, July 13 – Sept. 8, 1987–1991 (data set D + E)	8	0.10	0.001–0.92
(G) Himmerfjärd Bay, July 14 – Aug. 3, 1987–1991	5	0.69	0.002–0.97
(H) Himmerfjärd Bay, Aug. 15 – Sept. 5, 1987–1991	5	0.48	0.002–0.97
(I) Himmerfjärd Bay, July 14 – Sept. 5, 1987–1991 (data set G + H)	10	0.57	0.001–0.92
(J) Norrtälje area, Aug. 23 – Sept. 7, 1988–1992	5	0.03	0.002–0.97

weak correlation between acoustic abundances and catches, as the density of herring in commercial gillnet catches may be at least one order of magnitude higher than in our gill nets.

In the analyses of effects of Secchi disk depth and temperature on the gillnet catchability (eq. (9)), we found increased catches with increased temperature and decreased catches with increased Secchi depth (Table 1, regression models d–f). It was, however, only the Secchi depth that had a statistically significant effect on catchability.

Discussion

There have been relatively few studies in which catches of fish in passive fishing gears have been related to independently assessed fish abundance, although comparisons between trawl catches and hydroacoustic fish abundance are common (see Thorne 1983). Bannerot and Austin (1983) and Richards and Schnute (1986) found a relatively poor correlation between catch per unit effort and the fish population present when they compared handline catches with abundance observations from divers and from a submersible. In comparisons made across lakes, or within a lake or a sea coastal area across seasons, the correlation between hydroacoustic fish abundance and vertical gillnet catches may be poor (Rudstam et al. 1987, 1988). Within a lake, however, fairly good correlations between hydroacoustics and vertical gillnet catches have been found between years, provided that studies were done during the same month each year (Jacobson et al. 1990; Rudstam and Johnson 1992). For brown trout, Borgstrøm (1992) found a positive but nonlinear relationship between gillnet catches and mark–recapture abundance estimates. These relationships may vary between species. Ryan (1984) found a fairly strong positive relationship between fyke net catches and mark–recapture abundance estimates for brook trout (*Salvelinus fontinalis*) but not for Atlantic salmon (*Salmo salar*). For clupeids in coastal areas of the Baltic Sea, our results indicate a positive relationship, although the amount of variation in gillnets catches explained by hydroacoustic abundance is less than 30% even after correction of catches for both encounter rate and mesh selectivity (Table 1, regression model b).

If effects of transparency and possibly also temperature could be accounted for, an improved relationship between fish abundance and gillnet catches would be expected. To illustrate this, we performed a regression analysis with corrected gillnet catches as the dependent variable and the product of hydroacoustic fish abundance and the residual catchability coefficient as the independent variable:

$$(10) \quad CPUE_{corr} = a_{12} + a_{13} \cdot N_{sonar} \cdot Q.$$

The residual catchability coefficient was estimated only from the Secchi disk depth (model e in Table 1), since Secchi depth and temperature are strongly correlated (see below) and including effects of temperature improved the explanatory power of the model little (cf. models e and f in

Table 1). Adding the effects of Secchi depth on catches (eq. (10)) resulted in a regression model with an intercept not significantly different from zero and increased the determination coefficient between catches and fish abundance from 0.28 to 0.38 (models b and g, respectively, in Table 1).

That increased temperature led to higher catchability and increased Secchi disk depth led to lower catchability is consistent with our expectations. Fish activity or swimming speed decreases at lower temperatures. Increased transparency enhances the visibility of a net, resulting in an increase in net avoidance. It is also possible that activity increases at low transparency and visibility, as fish may have to feed for longer times or risk becoming more active (cf. Vinyard and O'Brien 1976; Wright and O'Brien 1984). The relative importance of temperature and visibility is difficult to evaluate because these parameters are correlated (Pearson correlation coefficient = -0.58, $p < 0.001$; SAS 1987*b*, pp. 259–284); however, the effect of Secchi depth was statistically significant, while the effect of temperature was not (Table 1, models d and e).

In our analyses, we combined acoustic abundance estimates and gillnet catches from different time periods and from many different coastal areas. Part of the poor relationship between gillnet catches and acoustic abundance could be due to specific attributes of sampling locations or seasonal changes in the vulnerability of fish to capture (cf. Jacobson et al. (1990) and Rudstam and Johnson (1992) as discussed above). To test this, we analysed the relationship between catches and acoustic abundance separately for three different areas: Himmerfjärd Bay, the Norrtälje area, and the Askö area. We also analysed two sets of data from several coastal stations, all sampled within 3 weeks. The analyses were performed with linear regressions between $CPUE_{corr}$ and hydroacoustic abundance (Table 2). Of the 10 resulting tests, only three (all from Himmerfjärd Bay) gave r^2 values larger than 0.28, which was the r^2 value derived when all data were included in the analysis. Thus, restricting the analysis to a short period of the year and a specific station does not necessarily improve the relationship between hydroacoustics and catches.

We did combine catches from two types of nets (1.5- and 3-m-wide nets) by multiplying catches in the 1.5-m nets by a factor of 2. If the fish are better at avoiding smaller than larger nets, this procedure may bias the results. We tested this in the following way. From the relationship between the hydroacoustic fish abundance and $CPUE_{corr}$ (model b in Table 1), expected $CPUE_{corr}$ values were calculated and compared with actual catches. From these comparisons, frequencies of "too-low" and "too-high" expected catches, as compared with actual catches, were registered separately for 1.5- and 3-m gill nets. The difference between these frequencies was evaluated with a χ^2 test with Yates' correction (Dixon and Massey 1969, pp. 240–243) and found to be clearly nonsignificant ($\chi^2 = 0.60$, df = 1, $p > 0.40$). This justifies the procedure of multiplying catches in 1.5-m gill nets by a factor 2 to make them compatible with catches in the 3-m gill nets.

As described under Materials and Methods, there were differences in the vertical coverage of gill nets and the acoustics. Gillnet catches represented the entire water column, while the upper 3 m and the deepest 1 m are not sampled with hydroacoustics. Restricting the analysis to only the 3–15 m depth interval did not, however, improve the relationship between catches and hydroacoustics. A possible explanation for this is that hydroacoustics gives a instantaneous description of the vertical distribution of fish, while the gill nets integrates over many hours, including periods of vertical migration.

A large proportion of the variance in gillnet catches not explained by fish abundance, fish size, gill net selectivity, or environmental factors such as transparency and temperature is likely to be a result of patchiness in fish distribution. Gill nets integrate catches over time but in a relatively small area whereas acoustics integrate abundance over a larger area but over a short time period. Increasing the total quantity of nets used in each survey could improve the relationship between catches and abundance by better integrating over spatially heterogeneous fish distributions. However, increasing sample size will not correct for the biases associated with temperature and Secchi disk depth observed in this study. This implies that changes in gillnet catches could be difficult to evaluate in relation to, for example, changes in temperature, eutrophication, or other environmental factors that influence water clarity (e.g., Cyrus and Blaber 1992; Hayes et al. 1992).

Finally, we would like to make two comments. First, the results presented in this paper are valid only for clupeids and possibly only for Baltic Sea conditions. For other species and areas, the relationship between gillnet catches and fish abundance may be stronger or weaker than suggested by our study. Second, we have used the term "acoustic fish abundance" to stress that the abundance estimated from sonar data might differ from the actual fish abundance. In a recent book on hydroacoustics, MacLennan and Simmonds (1992) reviewed comparisons between abundance estimates derived by different techniques. They concluded that hydroacoustics often gave better estimates than alternative methods. Similar results were also presented by Dahm et al. (1992). There are, however, relatively few studies in which the hydroacoustic fish abundances are compared with actual known abundances. Such comparisons are necessary to evaluate the conditions where hydroacoustics correctly estimates the abundance and where the behaviour of fish or other factors complicate these assessments.

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Efficiency and bias of estimators and sampling designs for determining length–weight relationships of fish

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Abstract: The parameters of the allometric equation used to describe the length–weight relationship in fish are usually estimated by linear regression of log-transformed data. Simulation of length–weight regressions showed that for sample sizes commonly encountered in fisheries research, estimates of the intercept are biased high. In contrast with this, estimates of mean weight-at-length are biased low. As a result, different bias-correction factors are necessary to adjust for transformation bias. When the objective is to estimate the intercept of the length–weight equation, two existing methods correct for transformation bias. The appropriate bias-correction factor for mean weight-at-length, however, is $\exp(\sigma^2/2)$ where σ^2 is the residual variance of the regression. As an alternative to the log-transformation method, the properties of nonlinear least-squares regression were explored. Estimates obtained with nonlinear regression are less efficient, but are relatively robust to departures from the assumed error structure. Simulation of several sampling designs showed that greater precision without loss of accuracy is obtained as subsampling is concentrated toward the extremes of the length distribution.

Résumé : Les paramètres de l'équation allométrique utilisés pour décrire la relation longueur–poids chez les poissons sont généralement estimés par régression linéaire des données après transformation logarithmique. La simulation des régressions longueur–poids a montré que pour les tailles d'échantillon communément observées dans la recherche sur les pêches, les estimations de l'ordonnée à l'origine renferment un biais élevé. Par contre, les estimations du poids moyen selon la longueur renferment un faible biais. En conséquence, il est nécessaire d'avoir recours à différents facteurs de correction pour tenir compte du biais dû à la transformation. Quand l'objectif est d'estimer l'ordonnée à l'origine de l'équation longueur–poids, deux méthodes permettent de corriger le biais dû à la transformation. Le facteur approprié de correction du biais pour le poids moyen selon la longueur est toutefois $\exp(\sigma^2/2)$ où σ^2 est la variance des résidus de la régression. Pour remplacer la méthode de transformation logarithmique, on a exploré les propriétés de la régression non linéaire par les moindres carrés. Les estimations obtenues par la régression non linéaire sont moins efficaces, mais sont relativement robustes si on l'éloigne de la structure d'errreurs posée au départ. La simulation de plusieurs plans d'échantillonnage a montré qu'on obtient davantage de précision sans perte d'exactitude lorsque le sous-échantillonnage est concentré vers les extrémités de la distribution des longueurs.

[Traduit par la Rédaction]

Introduction

Length–weight regressions are used extensively in fisheries science. Three of the primary applications are converting length measurements to weight estimates (Everhart and Youngs 1981), computing condition indices (Anderson and Gutreuter 1983), and representing the relationship between length and weight (LeCren 1951). The most common form of the length–weight relationship is the allometric equation (equation 1) that expresses weight (W) as a function of length (L):

$$[1] \quad W = A \cdot L^B \xi$$

where ξ is a lognormally distributed multiplicative error term. Usually, the parameters A and B are estimated by applying a logarithmic transformation to equation 1 to obtain the straight-line relationship

$$[2] \quad \ln(W) = \ln(A) + B \cdot \ln(L) + \epsilon$$

where $\epsilon = \ln(\xi)$ and is distributed as $N(0,\sigma^2)$. The parameters of this are then estimated by linear regression. Retransformation of equation 2 with the inverse logarithmic transformation returns the linearized equation to its original allometric form (e.g., Ricker 1975).

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Despite wide usage, several statistical properties of this estimation procedure remain unexplored in the context of fisheries applications. For example, estimates of A obtained as above are known to be asymptotically biased low by a factor of $\exp(\sigma^2/2)$ (e.g., Miller 1984; Gutreuter 1990) where σ^2 is the residual variance of the regression. This bias occurs because the use of the logarithmic transformation shifts the basis of the regression from the mean to the geometric mean (equivalent to the median in this case). For data drawn from a lognormal distribution, the ratio of the mean to the geometric mean is $\exp(\sigma^2/2)$. However, small sample sizes can also introduce bias to the estimate of A. Methods to correct for the small-sample bias in estimates of A have been described in the statistical literature (Finney 1951; Neyman and Scott 1960; Bradu and Mundlak 1970), but have not been applied to the problem of estimating the parameters of the allometric equation.

Nonlinear least-squares regression has been suggested as an alternative method to estimate the parameters of the allometric equation (Zar 1968; Glass 1969). This method, however, assumes an additive error term rather than a multiplicative error term. Specifically, nonlinear least-squares regression assumes

$$[3] \qquad W = A \cdot L^B + \epsilon$$

where ϵ is normally distributed and has constant variance. In this study, we found that neither of these assumptions applied. In many situations, however, the actual error structure is either unknown or cannot be differentiated from alternative error structures from the data. Therefore, our interest was to examine the robustness of nonlinear least-squares regression to violations of its assumptions. In particular, we examined the precision and bias of parameter estimates and predictions of weight-at-length given that the statistical model's assumptions were violated.

In addition to the estimator used, the design used to subsample fish has a substantial effect on the precision of estimates obtained (Ricker 1975). Often, far more fish are captured than can be sampled for individual measurements of length and weight. Little guidance is available regarding the efficiency of various subsampling designs. Ricker (1975) suggested that subsamples should be distributed evenly across the range of lengths and weights available, but gave no justification for this recommendation.

This study examines how the selection of the statistical estimator, sampling design, and sample size affects the precision and accuracy of length–weight equation parameter estimates and estimates of mean weight-at-length. To accomplish this, we simulated the process of sampling and estimating length–weight parameters from a population with known length–weight parameters. These simulations were based on observed length and weight measurements for haddock (*Melanogrammus aeglefinus*). A simulation approach enabled us to compare bias and to evaluate the efficiency of sampling designs and sample sizes with greater flexibility than empirical comparisons would permit.

Methods

Parameter values for this study were based on data collected during spring trawl surveys conducted by the Department of Fisheries and Oceans, Canada, on Georges Bank in 1984 and from 1986 to 1991. Methods used in these surveys are detailed in Gavaris (1989). Estimates of mean weight-at-length were obtained by linear regression using equation 2. Regressions were restricted to fish greater than or equal to 45 cm. A 45-cm minimum size was used to reduce problems associated with variability in weight measurements which was the case for smaller fish. The distribution of residuals was examined to determine the appropriate error structure for the simulations. A test for heteroscedasticity (White 1980; SAS Institute, Inc. 1989) was conducted to determine if variance after log-transformation was equal across all size classes.

Simulations were conducted using three sampling designs. In all simulations, fish lengths ranging from 35 to 75 cm were generated. A minimum length of 35 cm was chosen for the simulations because technological advances have recently made it possible for fish smaller than 45 cm to be weighed accurately at sea. In the first sampling design, an equal number of fish were generated in each length interval (uniform sampling). In the second sampling design, observations were generated in proportion to the length–frequency distribution from the research survey data (proportional sampling). In the final sampling design, an equal number of fish were generated in the smallest and largest length intervals (endpoint sampling). Sampling only the smallest and largest length intervals is motivated by the statistical literature that suggests that maximal precision in regression parameter estimates should be obtained when the variance of the independent variable is greatest. An example of the size distribution for each of these sampling designs for a sample size of 252 is given in Fig. 1. Sample sizes ranging from 36 to 252 in increments of 36 (i.e., N = 36, 72, 108, 144, 180, 216, and 252) and sample sizes of 504 and 1008 were simulated for each sampling design. A sample size increment of 36 was used because this was the number of samples allowing one fish per length interval to be simulated. Additional simulations with a larger sample size (N = 2016) were conducted for the uniform sampling design to examine the asymptotic properties of the estimators.

For each sampling design and sample size (N), 1000 sets of N length–weight observations were generated. Lognormally distributed weight-at-length observations, denoted as $W(L)$, were generated from

$$[4] \qquad W(L) = e^{(\mu + \sigma \cdot Z)}$$

where μ and σ were chosen so that

$$E(W) = e^{(\mu + \sigma^2/2)}$$

$$\mathrm{Var}(W) = e^{(2\mu + 2\sigma^2)} - e^{(2\mu + \sigma^2)}$$

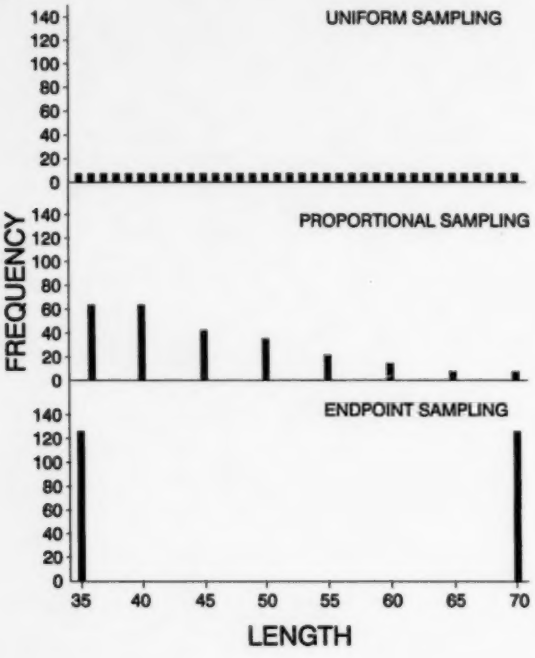
and where

$$Z \sim N(0,1).$$

The mean and variance of $W(L)$ were based on the mean and variance of weight-at-length observation from the surveys. Lengths were assumed to be measured without error.

After weight-at-length observations were generated, the parameters of the length–weight relationship were estimated using two methods: linear regression of the log-transformed data and nonlinear regression of the untransformed data.

Fig. 1. Size distribution of haddock for uniform, proportional, and endpoint sampling designs with $N = 252$.



We used the notation A and B to denote the parameters of the allometric model and B_0 and B_1 to denote the intercept and slope of the linear regression on log-transformed data.

With the log-transformation method, estimates of B from B_1 are unbiased when model assumptions (i.e., equal variance and normal distribution of log-transformed weights at length) are met (Miller 1984). However, estimates of A from the inverse transformation, here denoted as $\hat{a}_{T1} = \exp(B_0)$, are biased low (Miller 1984). To partially correct for this bias, Miller (1984) recommended the use of a low-bias estimator of A, denoted by $\hat{a}_{T2}$:

$$[5] \qquad \hat{a}_{T2} = e^{B_0}\, e^{\sigma^2/2}$$

where σ^2 is the residual variance in the regression. The low-bias method accounts for the shift in the basis of the regression from the arithmetic mean to the geometric mean when the log-transformation is applied (Goldberger 1968; Miller 1984; Gutreuter 1990).

Another method for correcting transformation bias was derived by Finney (1951) and was extended by Goldberger (1968) and Bradu and Mundlak (1970). Based on theoretical developments, Bradu and Mundlak's (1970) method produces an unbiased estimate of A, here denoted as $\hat{a}_{T3}$:

$$[6] \qquad \hat{a}_{T3} = e^{B_0}\, g_m(t)$$

where

$$g_m(t) = 1 + \sum_{p=1}^{\infty} \frac{m^p(m+2p)}{m(m+2)\ldots(m+2p)} \left(\frac{m}{m+1}\right) \frac{1}{p!}\, t^p$$

$$m = N - 2 \qquad \text{(degrees of freedom)}$$

$$t = \frac{m+1}{2m}\left(\sigma^2 - s^2(B_0)\right)$$

$$s^2(B_0) = \sigma^2\, \frac{1}{N}\, \frac{\bar{x}^2}{\Sigma(x-\bar{x})^2}.$$

The summation in $g_m(t)$ was performed over the first six elements because the contribution of additional terms was negligible.

An alternative method to produce an unbiased estimate of A, deonted by $\hat{a}_{T4}$, was developed by Neyman and Scott (1960):

$$[7] \qquad \hat{a}_{T4} = e^{B_0}\, e^{(\sigma^2 - \mathrm{Var}(B_0))/2}.$$

Neyman and Scott's (1960) method also adjusts for the shift from the arithmetic mean to the median, but approximates the effects of small sample sizes differently than Bradu and Mundlak's (1970) method.

Nonlinear least-squares estimates of length–weight parameters were computed with the Marquardt algorithm (Marquardt 1963; Ratkowsky 1983; SAS Institute, Inc. 1989). Initial values for the search were set at the known values. The Marquardt algorithm requires first partial derivatives with respect to the parameters. For the allometric equation, the vector of first partial derivatives, F, is

$$[8] \qquad F = \begin{bmatrix} \dfrac{\partial}{\partial A}(A \cdot L^B) \\ \dfrac{\partial}{\partial B}(A \cdot L^B) \end{bmatrix} = \begin{bmatrix} L^B \\ A \cdot L^B \cdot \ln(L) \end{bmatrix}.$$

Second partial derivatives are also needed for the calculation of bias estimates. The Hessian matrix, denoted by H, for the allometric equation is

$$[9] \qquad H = \begin{bmatrix} \dfrac{\partial^2(A \cdot L^B)}{\partial A^2} & \dfrac{\partial^2(A \cdot L^B)}{\partial A \partial B} \\ \dfrac{\partial^2(A \cdot L^B)}{\partial A \partial B} & \dfrac{\partial^2(A \cdot L^B)}{\partial B^2} \end{bmatrix} = \begin{bmatrix} 0 & L^B \cdot \ln(L) \\ L^B \cdot \ln(L) & A \cdot L^B \cdot \ln(L)^2 \end{bmatrix}.$$

Nonlinear regression parameter estimates are asymptotically unbiased (Ratkowsky 1983), but can be biased for small sample sizes. Predictions of parameter bias were obtained by the following (Ratkowsky 1983, equation 2.3):

$$[10] \qquad \text{Bias } \hat{P} = \frac{-\sigma^2}{2}\left[\sum_{u=1}^{N} FF'\right]^{-1} \sum_{t=1}^{N} F\, \mathrm{Trace}\left\{\left[\sum_{u=1}^{N} FF'\right]^{-1} H\right\}$$

where

$$\hat{P} = \begin{bmatrix} \hat{A} \\ \hat{B} \end{bmatrix}$$

and where F and H are evaluated at each data point indexed by u. We denoted estimates of A and B without bias adjust-

Table 1. Slope estimates for haddock simulations obtained using the log-transformation method ($\hat{b}_T$), unadjusted nonlinear regression ($\hat{b}_{N1}$), and nonlinear regression with bias adjustment ($\hat{b}_{N2}$). The actual value of B used in the simulations was 3.120. Results are based on 1000 simulations with N fish in each simulation. The mean is across all simulations and all sample sizes.

N	$(\hat{b}_T)$	$(\hat{b}_{N1})$	$(\hat{b}_{N2})$
36	3.122	3.128	3.125
72	3.118	3.127	3.126
108	3.119	3.117	3.116
144	3.119	3.121	3.121
180	3.121	3.119	3.119
216	3.119	3.120	3.120
252	3.120	3.120	3.119
504	3.119	3.120	3.120
1008	3.119	3.118	3.118
Mean	3.120	3.121	3.120

ments as $\hat{a}_{N1}$ and $\hat{b}_{N1}$, and with bias adjustments as $\hat{a}_{N2}$ and $\hat{b}_{N2}$.

For each parameter estimated, actual bias was computed as the difference between the mean of the parameter over the 1000 simulations and the known value used to generate the data. Precision was measured as the square root of the mean square error (RMSE) and was computed as (Cochran 1977)

$$[11] \quad \text{RMSE}(\xi) = \sqrt{\text{Bias}(\xi)^2 + \text{Var}(\xi)}.$$

Results and discussion

Parameter values for simulations

The mean weight (kilograms) at length (centimetres) for haddock from spring survey cruises on Georges Bank was

$$W = 6.59 \times 10^{-6} L^{3.120}.$$

The distribution of residuals of length–weight regressions from seven cruises indicated that log(W) at length was normally distributed (Fig. 2). For six of the seven cruises, tests for equality of residual variance supported the assumption of equal variance across length groups ($P > 0.05$). Residual variance (σ^2) averaged 0.0149, corresponding to a coefficient of variation of about 12% for mean weight-at-length.

Simulation results: comparison of estimation methods

Bias in the various quantities estimated and comparisons between methods of adjusting for bias are presented for the uniform sampling design. Similar results were obtained for the other sampling designs, since the sources of bias were the same.

As indicated by Miller (1984), $\hat{b}_T$ was unbiased across all sample sizes (Table 1). Estimates of $\hat{b}_{N1}$ had negligible bias across all sample sizes simulated and bias-adjusted estimates ($\hat{b}_{N2}$) had no detectable bias (Table 1). Although the log-transformation method and nonlinear regression both produced accurate (i.e., unbiased) estimates of B, the two methods differed substantially in the precision of their estimates. Across all sample sizes, the RMSE of slope estimates produced by the log-transformation method was about 40% less than that obtained by nonlinear regression (Fig. 3).

Fig. 2. Distribution of residuals from regressions of ln(W) against ln(L).

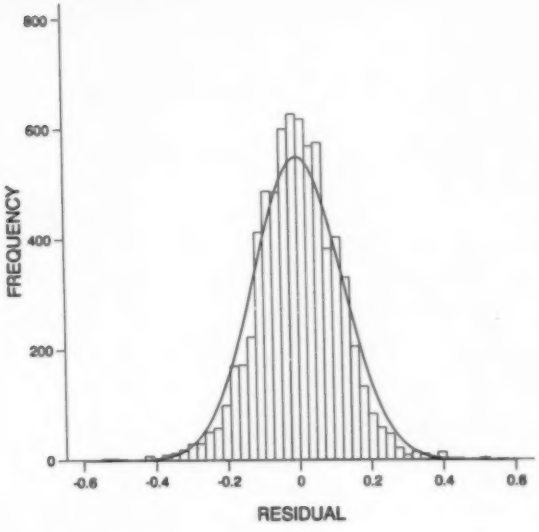


Fig. 3. Comparison of RMSE of estimates of (A) slope and (B) intercept of the allometric equation obtained with the log-transformation method and nonlinear regression for haddock, uniform sampling design. Each data point is based on 1000 simulations.

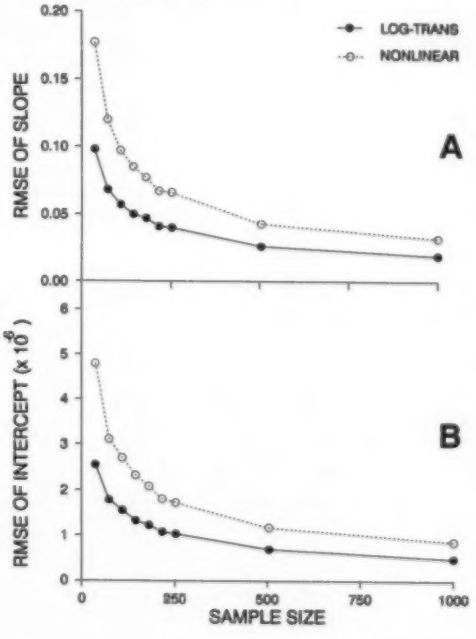


Table 2. Intercept estimates for haddock obtained using the log-transformation method and nonlinear regression. All entries are 10^{-6}. The actual value of A used in the simulations was 6.59×10^{-6}. Results are based on 1000 simulations with N fish in each simulation. The mean is across all simulations and all sample sizes.

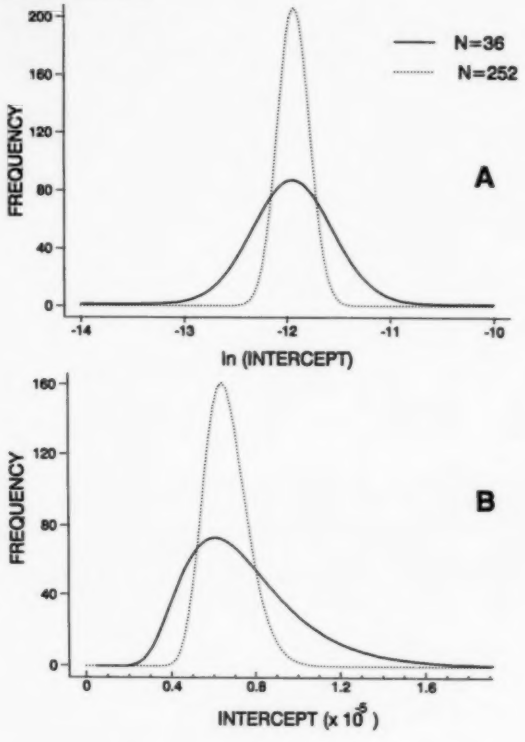
	Log-transformation method				Nonlinear regression	
N	$\hat{a}_{T1}$	$\hat{a}_{T2}$	$\hat{a}_{T3}$	$\hat{a}_{T4}$	$\hat{a}_{N1}$	$\hat{a}_{N2}$
36	6.98	7.03	6.50	6.50	8.10	6.52
72	6.82	6.87	6.61	6.61	7.16	6.43
108	6.75	6.81	6.63	6.63	7.20	6.71
144	6.69	6.74	6.61	6.61	6.95	6.60
180	6.62	6.67	6.56	6.56	6.93	6.65
216	6.65	6.70	6.61	6.61	6.82	6.59
252	6.62	6.67	6.60	6.63	6.83	6.62
504	6.60	6.65	6.61	6.61	6.69	6.59
1008	6.59	6.63	6.62	6.63	6.68	6.63
2016	6.54	6.59	6.58	6.59	—	—
Mean	6.68	6.73	6.59	6.60	7.04	6.59

Ricker (1973, 1975) recommended adjusting the slope estimate in length–weight regressions by the correlation coefficient to obtain the geometric mean regression. This suggestion is not commonly followed because correlation coefficients are generally high (i.e., >0.97; see Culver et al. 1985; Willis et al. 1991 for some examples) and adjustments to the slope inconsequential. Our results indicate that the use of the geometric mean regression is unnecessary if the assumption of negligible measurement error on length is met.

Estimates of A in the allometric equation using the log-transform method without adjustment ($\hat{a}_{T1}$) have been shown to be asymptotically biased low (Miller 1984; Gutreuter 1990). This assertion is theoretically sound, but in our simulations the asymptote was not met even for a sample size of 1008 (Table 2). Additional simulations indicate that at a sample size of 2016, $\hat{a}_{T1}$ averaged 6.54×10^{-6}, a value very close to the predicted asymptote. For sample sizes up to 500, $\hat{a}_{T1}$ was biased high, and increasingly so for sample sizes less than 180 (Table 2). Of the three methods for correcting for transformation bias, nearly equivalent results were obtained with Bradu and Mundlak's (1970) method ($\hat{a}_{T3}$) and Neyman and Scott's (1960) method ($\hat{a}_{T4}$; Table 2). For sample sizes commonly encountered in fisheries research and management (i.e., less than 250), corrections for asymptotic bias ($\hat{a}_{T2}$) increased bias compared with the simple inverse transformation estimate ($\hat{a}_{T1}$; Table 2).

An important point to make is that estimates of B_0 had the same central tendency for all sample sizes, even though the distributions of $\hat{a}_{T1}$ and $\hat{a}_{T2}$ differed in their central tendency (Fig. 4). This has important implications for comparisons between samples or populations. For example, if samples are taken from two populations, comparison of $\hat{a}_{T1}$ between these populations can lead to incorrect conclusions if the sample size differs substantially. The correct

Fig. 4. Distributions of (A) B_0 (i.e., ln(intercept)) in the log-transformation method and (B) of the uncorrected estimate of the intercept of the length–weight relationship ($\hat{a}_{T1}$) for haddock, uniform sampling design. Each line represents the results of 1000 simulations with the given sample size (N).



method for evaluating differences between populations is to use B_0 as the basis for comparison. Note, however, that inferences concerning B_0 are really testing differences in median response rather than mean response. If the assumption regarding equal variance between populations is correct, these tests will also apply to the mean, since the ratio between the mean and median will be equal.

Estimates of $\hat{a}_{N1}$ were biased high at small sample sizes and continued to show positive bias even for sample sizes as large as 1008 (Table 2). Bias-adjusted estimates ($\hat{a}_{N2}$) varied between sample sizes, but generally were centered on the actual value. As with slope estimates, intercept estimates produced by nonlinear regression had lower precision than estimates obtained by the log-transformation method (Fig. 3).

Estimates of the mean weight-at-length obtained using $\hat{a}_{T1}$ (the uncorrected log-transform estimate) were biased low by a negligibly small amount (Tables 3 and 4). Estimates using $\hat{a}_{T2}$ corrected for this bias for all sample sizes (Tables 3 and 4). When $\hat{a}_{T3}$ or $\hat{a}_{T4}$ was used, estimates of mean weight-at-length were biased low, with a higher degree of bias for smaller sample sizes (Tables 3 and 4). Thus, the adjustment factor that correctly accounted for bias in parameter estimates (i.e., $\hat{a}_{T3}$ or $\hat{a}_{T4}$) was inappropriate for correcting for

Table 3. Predicted mean weight (g) at 35 cm for haddock using the log-transformation method and nonlinear regression. The actual mean weight at 35 cm in the simulated population was 433 g. Results are based on 1000 simulations with N fish in each simulation. The mean is across all simulations and all sample sizes.

	Log-transformation method			Nonlinear regression	
N	$\hat{a}_{T1}$	$\hat{a}_{T2}$	$\hat{a}_{T3}$ and $\hat{a}_{T4}$	$\hat{a}_{N1}$	$\hat{a}_{N2}$
36	429	433	400	432	342
72	430	433	417	432	385
108	430	433	422	434	403
144	430	433	425	433	410
180	430	433	426	433	415
216	430	433	427	433	417
252	430	433	428	433	420
504	430	433	431	433	426
1008	430	433	432	433	430
Mean	430	433	423	433	405

Table 4. Predicted mean weight (g) at 70 cm for haddock using the log-transformation method and nonlinear regression. The actual mean weight at 70 cm in the simulated population was 3764 g. Results are based on 1000 simulations with N fish in each simulation. The mean is across all simulations and all sample sizes.

	Log-transformation method			Nonlinear regression	
N	$\hat{a}_{T1}$	$\hat{a}_{T2}$	$\hat{a}_{T3}$ and $\hat{a}_{T4}$	$\hat{a}_{N1}$	$\hat{a}_{N2}$
36	3737	3765	3483	3767	2966
72	3735	3763	3619	3768	3385
108	3736	3764	3668	3763	3489
144	3736	3764	3692	3765	3561
180	3738	3766	3708	3764	3600
216	3732	3760	3712	3761	3624
252	3737	3765	3723	3764	3647
504	3736	3764	3744	3765	3706
1008	3733	3761	3751	3761	3731
Mean	3736	3764	3678	3764	3520

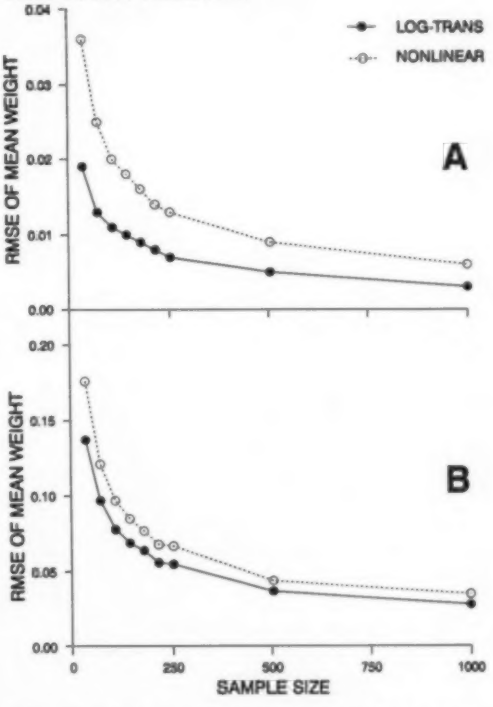
bias in predicted mean weight-at-length. These results are important, since they show that the appropriate bias-correction factor depends on how the length–weight equation will be used. When length–weight regressions are used to convert length measurements to weight estimates, $\hat{a}_{T2}$ (i.e., $\exp(\sigma^2/2)$) is the appropriate correction factor. However, if the intent is to represent the relationship between length and weight, or to compute a condition factor based on the intercept of the length–weight equation, then the appropriate bias-correction factor is $\hat{a}_{T3}$ or $\hat{a}_{T4}$.

Estimates of the mean weight-at-length using $\hat{a}_{N1}$ and $\hat{b}_{N1}$ showed no detectable bias (Tables 3 and 4). When $\hat{a}_{N2}$ and $\hat{b}_{N2}$ were used, mean weight-at-length was underestimated, with a greater degree of bias for small sample sizes (Tables 3 and 4). Thus, as with the log-transformation method, adjustments made to correct for bias in parameter estimates were inappropriate for adjusting mean weight-at-length estimates. Similar to slope and intercept estimates, estimates of mean weight-at-length obtained with nonlinear regression had lower precision than those from the log-transformation method (Fig. 5).

Simulation results: comparison of sampling designs

The endpoint sampling design was most efficient for nearly all quantities estimated, producing the lowest RMSE for a given sample size for both the log-transformation and nonlinear regression methods (Figs. 6 and 7). The only quantity for which endpoint sampling was not clearly more efficient was predicted mean weight at 50 cm where uniform sampling had similar efficiency (Table 5). The greater efficiency of endpoint sampling is predictable from regression theory which holds that the highest precision in linear least-squares estimation occurs when the contrast (i.e., the variance) of the independent variable is greatest (Neter and Wasserman 1974). This result is invariant to the logarithmic transformations because the maximum contrast

Fig. 5. Comparison of the RMSE of estimates of mean weight at (A) 35 cm and (B) 70 cm obtained with the log-transformation method and nonlinear regression for haddock, uniform sampling design. Each data point is based on 1000 simulations.



when the data are log-transformed also occurs with an equal sample size at the two extremes. Intuitively, it seems that designing sampling to mimic a two-point regression

Fig. 6. Comparison of precision (RMSE) of estimates of the intercept of the length–weight relationship for haddock using uniform, proportional, and endpoint sampling designs with the (A) log-transformation method and (B) nonlinear regression. Each data point is based on 1000 simulations.

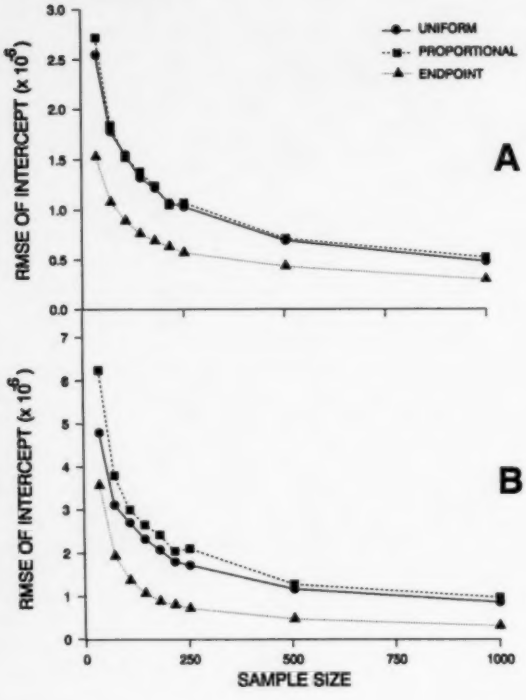
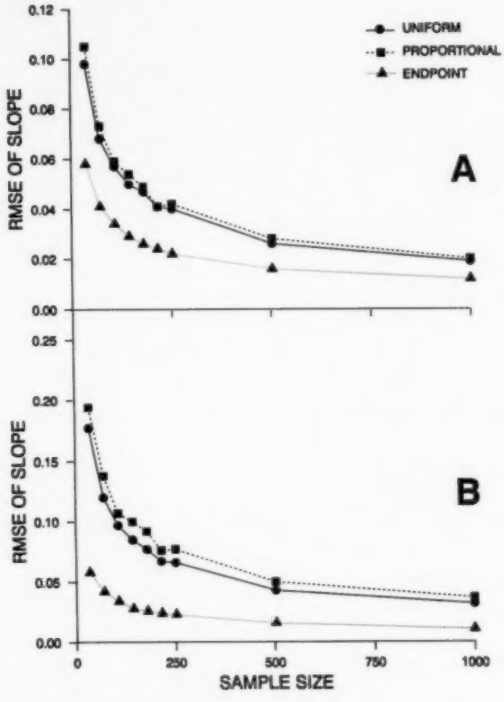


Fig. 7. Comparison of precision (RMSE) of estimates of the slope of the length–weight relationship for haddock using uniform, proportional, and endpoint sampling designs with the (A) log-transformation method and (B) nonlinear regression. Each data point is based on 1000 simulations.



would result in biased results. Statistical theory, however, indicates that regression experiments designed as two-point regressions are in fact optimal when the underlying relationship is linear, and that the results obtained are unbiased (Neter and Wasserman 1974).

After endpoint sampling, uniform sampling was generally most efficient, followed by proportional sampling (Figs. 6 and 7). Examination of the sums of squares for $\ln(L)$ explained the difference in efficiency of these sampling designs; greater variance (and contrast) in $\ln(L)$ was obtained with uniform sampling relative to proportional sampling. This result indicates that the relative efficiency of various sampling designs can be evaluated a priori by examination of the variance of $\ln(L)$.

In practical situations, the endpoint sampling design would rarely if ever be feasible because there are typically few samples available from the extremes of the length distribution. We suggest that while this sampling design cannot be strictly implemented in practice, it illustrates that greater precision in length–weight relationships can be obtained by emphasizing sampling effort at sizes near the ends of the length–frequency distribution. Thus, sampling protocols such as "sample 50 fish smaller than x cm and 50 fish larger than y cm for length and weight" could be implemented. Such a strategy is relatively simple to execute in field situations, and estimates obtained would approach the precision theoretically obtainable with strict endpoint sampling.

Biological considerations

In addition to the statistical considerations for sampling designs explored in this paper, several biological factors must also be considered. Perhaps foremost is the need to be able to test the assumption that the data measured follow the allometric equation. If lengths and weights are collected across growth stanzas, different length–weight relationships may be evident (Ricker 1975). In such a situation, a single allometric equation would be inadequate for describing the length–weight relationship. With strict endpoint sampling, however, it would not be possible to test for nonlinearity in the regression fit. Thus, if there is concern that the samples collected represent more than one growth stanza, the sampling design should be modified to allow for statistical tests of lack of fit (e.g., Neter and Wasserman 1974).

Another important consideration for sampling designs is the fact that the fish collected are often used for multiple biological measurements. For example, fish are often collected to determine length–frequency, age composition, or diet composition. As such, the optimal sampling designs for

Table 5. Standard deviations of mean weight-at-length estimates. Standard deviations are compared for fish of 35, 50, and 70 cm and for the uniform (U), proportional (P), and endpoint (E) sampling designs. Results are based on 1000 simulations with N fish in each simulation.

	35 cm			50 cm			70 cm		
N	U	P	E	U	P	E	U	P	E
				Log-transformation method					
36	0.019	0.014	0.013	0.027	0.033	0.028	0.137	0.200	0.110
72	0.013	0.010	0.009	0.019	0.023	0.018	0.097	0.139	0.074
108	0.011	0.008	0.007	0.015	0.019	0.016	0.078	0.113	0.064
144	0.010	0.007	0.006	0.014	0.016	0.013	0.069	0.102	0.055
180	0.009	0.006	0.005	0.012	0.015	0.012	0.064	0.094	0.050
216	0.008	0.005	0.005	0.011	0.013	0.011	0.056	0.078	0.045
252	0.007	0.005	0.005	0.010	0.013	0.011	0.055	0.079	0.040
504	0.005	0.004	0.004	0.007	0.009	0.008	0.037	0.054	0.031
1008	0.003	0.003	0.002	0.005	0.006	0.005	0.028	0.039	0.021
				Nonlinear regression					
36	0.036	0.030	0.012	0.038	0.033	0.027	0.176	0.282	0.106
72	0.025	0.021	0.009	0.026	0.023	0.019	0.121	0.199	0.078
108	0.020	0.016	0.007	0.021	0.019	0.015	0.097	0.155	0.064
144	0.018	0.015	0.006	0.019	0.017	0.013	0.085	0.146	0.053
180	0.016	0.014	0.006	0.017	0.015	0.012	0.077	0.135	0.049
216	0.014	0.012	0.005	0.015	0.013	0.011	0.068	0.110	0.044
252	0.013	0.012	0.005	0.014	0.013	0.010	0.067	0.112	0.040
504	0.009	0.008	0.003	0.010	0.009	0.007	0.044	0.074	0.030
1008	0.006	0.005	0.002	0.007	0.006	0.005	0.035	0.056	0.021

these objectives need to be considered in formulating the overall sampling strategy. Also of pragmatic importance is that fish are often collected in groups, resulting in a cluster sample rather than a simple random sample (Cochran 1977). When this is the case, the sampling design also needs to take into account the variability among clusters (i.e., individual collection sites) as well as the variation among individual fish.

Conclusions

Although the results we present for nonlinear regression are specific to the allometric model, the statement by Ratkowsky (1983) that nonlinear regression in general produces biased parameter estimates was supported by our findings. The method he presented for predicting bias in parameter estimates effectively indicated the bias in our simulations, however. For the allometric model, nonlinear regression (with appropriate bias corrections) appears to be relatively robust to departures from its underlying assumptions (i.e., constant variance and normally distributed error terms). Bias-adjusted parameter estimates and predictions of mean weight-at-length showed no additional biases due to violations of assumptions. As expected, however, the precision of these estimates was lower than for the log-transformation method where the error structure was correctly specified. In actual applications, the decision as to which method to use should be based on theoretical considerations of the error structure as well as an analysis of residuals to determine departures from model assumptions.

The results we obtained for the log-transformation method indicate that either Bradu and Mundlak's (1970) method ($\hat{a}_{T3}$) or Neyman and Scott's (1960) method ($\hat{a}_{T4}$) should be used for estimating the parameters of the allometric model when the goal is to represent the relationship between length and weight. Although the two methods are based on different theoretical grounds, in practice they produce equivalent results. Because of the computational simplicity of Neyman and Scott's (1960) method relative to Bradu and Mundlak's (1970) method, we suggest the use of Neyman and Scott's (1960) method. When the model is used for predicting mean weight-at-length, $\hat{a}_{T2}$ should be used. In our simulations, the magnitude of bias was relatively small. This was particularly true for estimates of mean weight-at-length where the bias was less than 1%. It is important to note that for Georges Bank haddock, the variance of weight-at-length was also small, with about a 12% coefficient of variation. Examination of equation 6 shows that the degree of bias increases with increasing variance, however. As an example, we computed the value of $g_m(t)$ for the uniform sampling case, but with coefficients of variation of 25, 50, and 100% rather than the 12% actually observed (Table 6). Clearly, higher variance produces greater bias in estimates of A. Although coefficients of

Table 6. Values of $g_m(t)$ for assumed coefficients of variation (CV) of 25, 50, and 100% for a uniform sampling design. The value of $g_m(t)$ is the expected ratio of the actual intercept value to the mean value of $\hat{a}_{TI}$, the unadjusted intercept value obtained by the log-transformation method.

	$g_m(t)$		
N	25% CV	50% CV	100% CV
36	0.749	0.338	0.228
72	0.880	0.623	0.226
108	0.928	0.758	0.421
144	0.952	0.836	0.572
180	0.968	0.886	0.686
216	0.978	0.921	0.774
252	0.985	0.947	0.844
504	1.008	1.029	1.092
1008	1.019	1.073	1.243
2016	1.025	1.095	1.326

variation for weight-at-length may not reach such high levels, variability in other applications using logarithmic transformation (e.g., catch-per-unit-effort analysis) may approach or exceed the variance used in our examples.

As a final comment, biases in parameter estimates are not limited to the allometric model and logarithmic transformation (Neyman and Scott 1960). Generally, retransformed parameter estimates may be biased whenever a nonlinear, monotonic transformation is applied. As with the logarithmic transformation, the magnitude of such bias varies with sample size and underlying variance. As such, the appeal to asymptotic properties for estimating bias may be inappropriate or misleading. We suggest that careful attention is required whenever nonlinear transformations are used for parameter estimation.

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Factors associated with interannual and intraannual variation in nutrient limitation of phytoplankton growth in Castle Lake, California

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Abstract: In a 3-year study of Castle Lake, California, potential nutrient (N,P) limitation of phytoplankton growth occurred rapidly (within 1–4 d of ice-out). Both N and P acted as potential limiting factors to phytoplankton growth in short-term (4–5 d) bioassays. Phytoplankton responded strongly to single additions of N or P in 1990 and 1992 but weakly so in 1991. This difference was associated with low inorganic N concentrations during spring 1991. In 1990 and 1991, variation of the primary limiting element correlated with the N:P ratio of the zooplankton community; phytoplankton tended to be N limited when the zooplankton was dominated by species with high N:P ratios (*Diaptomus novamexicanus* and *Diacyclops thomasi*: N:P ratios, by mass = 10.6–12.5) but limited by P when low N:P taxa (*Daphnia rosea*, N:P = 4.7) dominated. However, N vs. P limitation and zooplankton elemental data for 1992 did not fit the 1990–1991 pattern and there was no correlation for the 3-year data set. A field experiment demonstrated that the inorganic N:P ratio (NH_4/SRP) increased dramatically with elevated *Daphnia* grazing but declined significantly with increased *Diaptomus*; this supported the 1990–1991 correlation between phytoplankton N/P limitation status and zooplankton community elemental ratio.

Résumé : Dans une étude menée pendant trois ans au lac Castle (Californie), on a observé que la limitation par les nutriants (N,P) de la croissance phytoplanctonique se produisait rapidement (1 à 4 jours après la disparition de la glace). Aussi bien N que P ont joué un rôle de facteurs limitants potentiels pour la croissance du phytoplancton dans des bioessais de courte durée (4–5 jours). Le phytoplancton a fortement réagi à des ajouts uniques de N ou de P en 1990 et 1992, mais faiblement en 1991. Cette différence était associée à de faibles concentrations de N inorganique au printemps 1991. En 1990 et 1991, la variation de l'élément limitant primaire était corrélée avec le rapport N:P de la communauté zooplanctonique; le phytoplancton tendait à être limité par N quand le zooplancton était dominé par des espèces présentant des rapports N:P élevés (*Diaptomus novamexicanus* et *Diacyclops thomasi*; rapports N:P, en masse = 10,6–12,5), mais il était limité par P quand dominaient des taxons ayant un faible rapport N:P (*Daphnia rosea*, N:P = 4,7). Toutefois, les résultats sur la limitation par N ou P et les données sur les éléments dans le zooplancton recueillies pour 1992 ne correspondaient pas au profil de 1990–1991, et il n'y avait pas de corrélation pour la série de données des trois années. Une expérience menée sur le terrain a démontré que le rapport N:P inorganiques (NH_4/PRS) augmentait de façon spectaculaire quand le broutage par *Daphnia* était élevé, mais baissait nettement avec une augmentation de la présence de *Diaptomus*; cela étayait la corrélation de 1990–1991 entre la limitation par N/P dans le phytoplancton et le rapport des éléments dans la communauté zooplanctonique.

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Introduction

The perspective afforded by long-term data sets has created an appreciation for the magnitude of interannual variability in ecosystems (Likens 1989). Analyses of long-term data have generated many questions regarding the physical, chemical, and biological factors that drive variability in ecosystem processes. For example, in lakes and oceans, interannual variation in the intensity of vertical water column mixing can alter the amounts of limiting nutrients returned to the euphotic zone and made available for phytoplankton production during the growing season (Harris 1986; Goldman et al. 1989). Biological factors affecting interannual variation in ecosystems include food web mediated processes that alter processes such as primary productivity and nutrient recycling via the indirect effects of coupled predator–prey interactions (Carpenter et al. 1985; Elser et al. 1988). The interplay of external factors and internal biotic interactions affecting patterns of ecosystem variation was highlighted by recent analyses of the 30-year primary productivity data for Castle Lake, California, in which distinct interannual and intraannual patterns of productivity were identified and found to be correlated with distinctly different causal factors (Jassby et al. 1990). As in Jassby et al.'s (1990) study, most attention regarding variation in ecosystem processes has focused on parameters such as producer biomass and primary productivity. Much less attention has been paid to other aspects of ecosystem function. For example, little is known about how patterns in nutrient availability and nutrient limitation vary on both interannual and intraannual time scales because most studies of phytoplankton growth limitation in lakes have been performed too sporadically to effectively characterize such patterns (Elser et al. 1990). However, it is the turnover of limiting nutrients that permits sustained energy transfer in ecosystems. Thus, understanding variation in productivity may ultimately require a more complete understanding of underlying patterns of nutrient cycling and nutrient limitation.

Like productivity variation, variation in nutrient availability is also a function of processes both external and internal to the lake. The association between the external loading of limiting nutrients (especially nitrogen (N) and phosphorus (P)) and limitation of phytoplankton abundance and growth is strongly established (Vollenweider 1976; Schindler 1977; Goldman 1981). Thus, variation in the amounts of limiting nutrients introduced to the lake either via inflows from the watershed or from wintertime accumulation of internally regenerated nutrients will produce variation in the potential phytoplankton production during the subsequent growing season. Evidence supporting the hypothesis that nutrient availability is also influenced by biotic interactions during the growing season itself has been increasing (Sarnelle 1992; Carpenter et al. 1993; Urabe 1993) since the initial evidence that changes in food web structure alter the relative availability of N and P was published (Elser et al. 1988). The mechanism by which food web interactions potentially alter N/P availability is also beginning to become established (Sterner et al. 1992). When zooplankton assemblages are dominated by taxa with low body N:P ratios (such as *Daphnia*: Andersen and Hessen 1991), phytoplankton growth is more likely to be limited by P because NH_4 is disproportionately recycled by the zooplankton. Conversely, zooplankton assemblages dominated by animals with high body N:P ratios (such as calanoid copepods) will disproportionately release P, enhancing N limitation in the algae. However, field studies linking patterns of variation in phytoplankton N vs. P limitation to the N:P ratios of consumers are lacking.

In this paper, we report the results of a 3-year study designed to characterize interannual and intraannual patterns of nutrient availability and limitation of phytoplankton growth in oligotrophic Castle Lake, California. We wished to evaluate the extent to which variation in nutrient limitation was associated with differences in the relative amounts of limiting nutrients available due to loading and regeneration prior to the growing season versus with differences in zooplankton-mediated recycling of nutrients within the growing season. To evaluate variation in initial inputs of limiting nutrients, we performed intensive monitoring and experimental studies immediately prior to and following ice break-up as well as through the establishment of thermal stratification. In particular, we wished to determine the earliest occurrence of potential nutrient limitation of phytoplankton growth in relation to variation in the winter/spring accumulation of phytoplankton nutrients and the timing of their subsequent depletion. Finally, we monitored crustacean zooplankton abundance during the study period and determined the N:P ratios of dominant taxa to test the hypothesis that shifts in N/P limitation were associated with the dynamics of elementally contrasting zooplankton taxa. We also performed a field experiment using two dominant Castle Lake zooplankton species with contrasting N:P ratios to determine whether inorganic N and P availability was affected by differential nutrient recycling by these species.

Methods

Study site

Castle Lake is a relatively small (20 ha), deep (35 m maximum depth), subalpine lake located in the Siskiyou mountains of northern California, USA (41°13′N, 122°22′W) at an elevation of 1657 m. The lake is mesooligotrophic (summer total phosphorus approximately 10 μg/L, summer chlorophyll *a* 2–7 μg/L) and has relatively soft water (dissolved inorganic carbon approximately 2.5–8 mg/L). Algal growth is often nutrient limited (Goldman and DeAmezaga 1984). Spring phytoplankton are dominated by diatoms and other chrysophytes, while dinoflagellates, gelatinous green and blue-green algae, and small flagellates characterize the summer phytoplankton community (Janik 1988; Elser 1992). The zooplankton of Castle Lake has been dominated historically by *Daphnia rosea*, *Diaptomus novamexicanus*, and *Holopedium gibberum* (Janik 1988; Jassby et al. 1990). However, in fall 1988, the lake was subjected to a whole-lake food web manipulation when the annual stocking of the dominant vertebrate planktivore (rainbow trout, *Oncorhynchus mykiss*) was discontinued. This manipulation had substantial effects on fish populations, zooplankton community structure, and phytoplankton dynamics in subsequent years (1989–1991). The main

result with respect to the crustacean zooplankton community has been a reduction in the abundance of *D. rosea* and *D. novamexicanus* (relative to years prior to 1989) and an increase in the abundance of the cyclopoid copepod *Diacyclops thomasi* (Elser et al. 1995). Rainbow trout stocking was reinstated in the fall of 1991. However, the responses of the Castle Lake plankton assemblages to the return of rainbow trout have not yet been fully analyzed.

Routine sampling and analyses

During 1990–1992, Castle Lake was sampled year-round, with sampling frequency changing according to season. During fall and winter, the lake was sampled approximately monthly. Immediately after ice-out, the sampling frequency was increased to twice per week until pronounced water column thermal stratification was established. During the rest of the growing season (summer, early fall), the lake was sampled weekly. Samples were taken from a fixed sampling raft at the deepest site, unless ice conditions prevented access.

On each date, temperature profiles were made using a YSI temperature–oxygen meter. Water samples were collected from fixed depths (0, 3, 5, 10, 15, 20, 25, 30, and 32 m) using a Van Dorn sampler. Water was passed through an 80-μm screen to remove large zooplankton and returned to the lakeside laboratory for analysis. NO_3-N and NH_4-N samples were prefiltered through GF/C filters and analyzed on the same day or refrigerated and analyzed within 48 h. SRP was measured in GF/C-filtered samples, often on the same day as collection in 1991 or on frozen samples within 3–5 weeks of collection in 1990 and 1992. NO_3-N was analyzed using the hydrazine reduction method (Kamphake et al. 1967), NH_4-N via the phenol–hypochlorite method (Solorzano 1969), and soluble reactive phosphorus (SRP) via the acid molybdate technique (APHA 1992).

Zooplankton were sampled at weekly intervals using a 12-L clear Schindler sampler fitted with 85-μm-mesh netting. Three Schindler trap samples from three depths evenly spaced in the epilimnion (generally 0.5, 3, and 5 m) were taken and pooled to form a single composite sample. Zooplankton in Castle Lake are strongly migratory (Redfield and Goldman 1978), so samples were taken both during the day (usually between 11:00 and 13:00 Pacific standard time (PST)) and at night (usually between 20:00 and 00:00 PST). Analyses involving zooplankton data were conducted using averages of the night and day samples. Samples were enumerated and 25–50 individuals of the dominant crustacean species were measured for estimation of dry mass.

Samples for elemental analysis were prepared for three dominant zooplankton species: *D. rosea*, *D. novamexicanus*, and *D. thomasi*; these samples were prepared once during late summer for each species. Studies by Andersen and Hessen (1991) indicate that seasonal variation in body elemental contents is small within species compared with strong interspecific differences. Replicates ($n = 3$) for analysis of N or P were prepared for each species by placing 80–150 animals (depending on body size) on precombusted, preweighed GF/C filters. Filters with animals were gently dried and kept frozen with desiccant until being reweighed and analyzed for N or P content. P content was determined by repeated persulfate oxidation followed by analysis of orthophosphate using the acid molybdate technique (APHA 1992), while N was determined using a Perkin-Elmer model 2400 elemental analyzer.

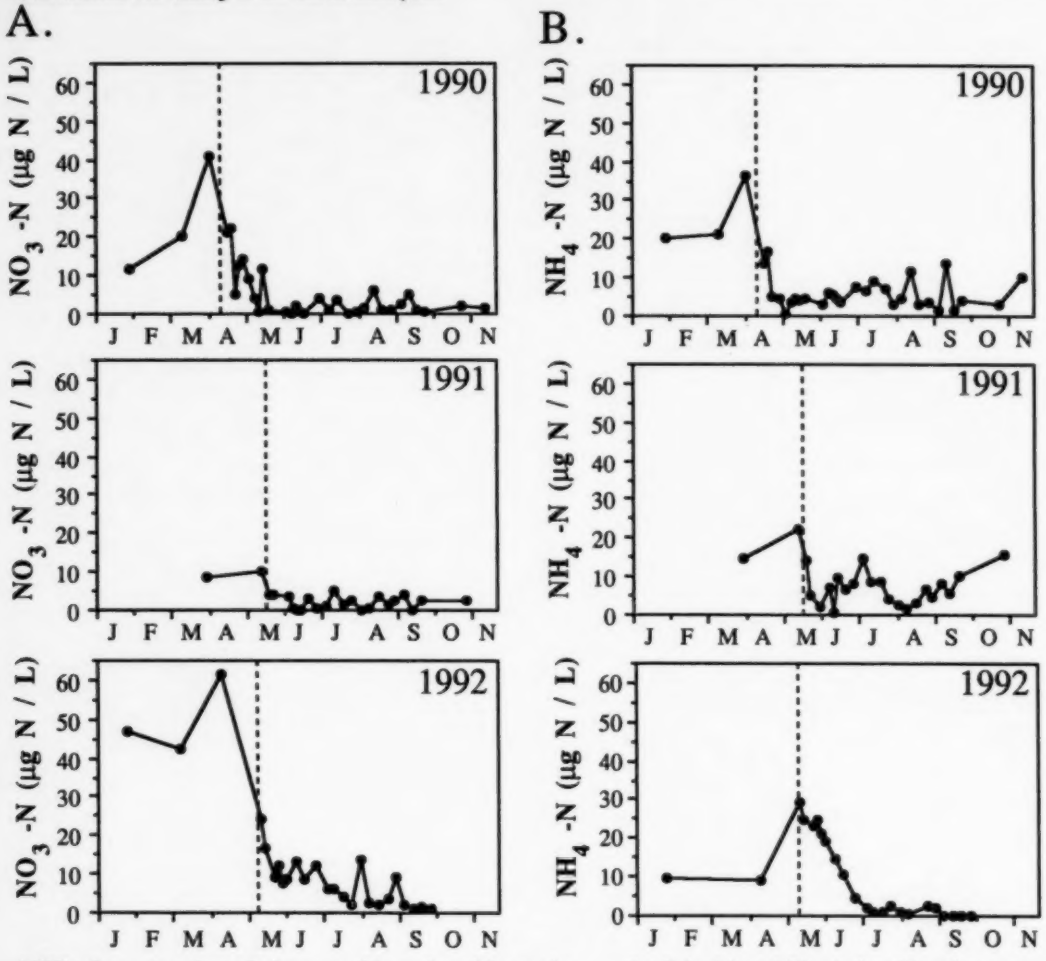
On 11 September 1992, a field experiment to examine the potential differential effect of elementally contrasting zooplankton species on nutrient availability was initiated. Twenty-six translucent polyethylene containers (1-L volume) were filled with epilimnetic water screened through 85-μm Nitex netting to remove crustacean zooplankton. Previous experiments in Castle Lake have shown that 85-μm mesh is sufficient to remove >90% of the crustacean zooplankton biomass without major impacts on larger phytoplankton taxa (Janik 1988). Individuals of *D. rosea* or *D. novamexicanus* were segregated by hand into small beakers containing filtered lake water and held in a refrigerator. Aliquots of isolated animals were then added to 1-L containers to achieve densities of 4, 8, 16, and 32 animals/L, in triplicate, for each species. Two 1-L containers did not receive any additions of animals. The experiment was incubated at a depth of 30% light penetration for 6 d. At the end of the experiment, animals were screened from containers and checked for viability (survival generally exceeded 90%) and water samples from each container were filtered through a GF/F filter for analysis of SRP and NH_4-N as described above.

Enrichment bioassays

To evaluate the potential for phytoplankton growth limitation by N vs. P, we generally used full factorial enrichment experiments involving PO_4, NH_4, and NO_3 (see below for changes in 1992). To characterize the time scales at which potential nutrient limitation changes, especially during the dynamic period immediately following ice-out, we executed enrichment experiments at weekly intervals for the first 4–6 weeks of the ice-free season in each year. Once thermal stratification had become strongly established, experiments were performed at approximately biweekly intervals.

Lake water (with phytoplankton) was collected from several depths in the epilimnion (during spring mixing when there was no thermal stratification, three arbitrary depths near the surface were chosen) and homogenized in a large container after screening through 80-μm Nitex. Samples for analysis of initial chlorophyll concentration were taken. Experimental bottles (acid-washed, 500-mL clear PVC) were then filled. Each treatment or treatment combination was executed in triplicate (1990–1991) or quadruplicate (1992). Experimental enrichments were generally approximately 1.5 μM PO_4-P, 22.5 μM NH_4-N, and 22.5 μM NO_3-N (N:P, 15:1 by moles) in appropriate treatments. However, in the first two experiments in 1990, NH_4 and NO_3 enrichments were approximately 11.25 μM in treatments that received *both* NH_4 and NO_3 (i.e., NH_4/NO_3 and $NH_4/NO_3/PO_4$). After enrichment, bottles were incubated in situ at 1.5 m depth (approximately the depth of 50% light penetration) for 4–5 d. Daily shaking and wave action served to reduce algal settling. After the incubation period, bottles were shaken and a subsample from each bottle was filtered onto a GF/F filter and kept frozen for later analysis of chlorophyll (corrected for pheophytin) using the fluorometric method (Strickland and Parsons

Fig. 1. Dynamics of inorganic N in Castle Lake surface waters during 1990–1992. (A) NO_3-N; (B) NH_4-N. Vertical broken lines indicate the timing of ice-out in each year.



1972) after extraction of pigment with methanol by soaking filters in the dark for 24 h at 6°C (Marker et al. 1980).

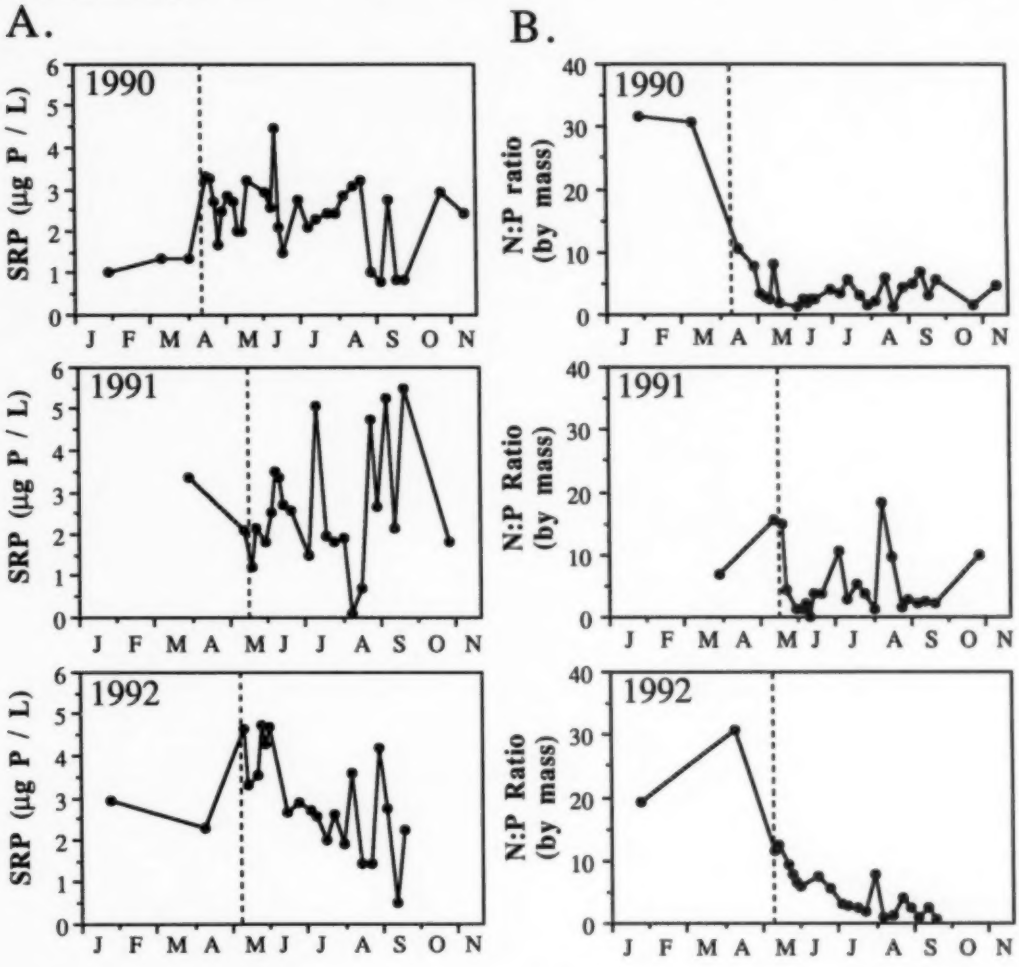
Ten nutrient enrichment experiments were conducted in 1990; each of these involved a complete factorial design with PO_4, NH_4, and NO_3 enrichment as treatments. In 1991, eight enrichment experiments were performed; six of these were full three-factor designs and two (late July and mid-August) were two-factor (PO_4 and NH_4) designs. During the mid-August experiment in 1991, the combined NH_4 /PO_4 treatment was lost. Eleven experiments were completed in 1992. Because the results of 1990 and 1991 experiments indicated little difference in the effects of NH_4-N and NO_3-N on phytoplankton growth (see below), the full three-factor design was reduced to a two-factor design with NH_4 and PO_4. However, enrichments of NO_3 alone were also applied in order to have a standardized set of data for growth response to N amendment in all years.

Data analysis

To characterize physical conditions during the seasonal cycles encompassed by our study period, we determined the Schmidt stability index (*S*, units grams per metre per square metre; Schmidt 1928) on each sampling date by calculating water density at each depth on the basis of depth and temperature using the International Equation of State (Millero and Poisson 1981) and applying the Schmidt equation using the hypsographic data for the lake. Because our enrichment experiments involved phytoplankton taken from the surface mixed layer, we summarized concentrations of SRP, NO_3-N, and NH_4-N by calculating mixed-layer averages for each parameter on each sampling date.

Enrichment bioassay data were analyzed in several ways. The main response variable for statistical analysis was final chlorophyll concentration. All experiments were individually analyzed by ANOVA. While final chlorophyll concentration was used in statistical analyses, graphical interpretation of the data was facilitated by considering the final chlorophyll concentrations in various treatments relative to the unenriched controls. Therefore, we calculated the ratio of mean final chlorophyll concentration in a given enrichment treatment to the mean chlorophyll in the controls for that experiment.

Fig. 2. Dynamics of soluble reactive phosphorus (SRP) and inorganic N:P ratio in the Castle Lake surface waters during 1990–1992. (A) SRP; (B) inorganic N:P ratio, by mass ((NH_4-N + NO_3-N) / SRP). Vertical broken lines indicate the timing of ice-out in each year.



Total dry mass and species-specific dry mass concentrations of zooplankton were determined using length–mass regressions specific for each of the crustacean taxa in the lake (Redfield 1980; Downing and Rigler 1986). To examine associations between shifts in in situ N/P limitation and changes in zooplankton elemental stoichiometry in the lake, we calculated the mean community N:P ratio of the zooplankton for the date closest to the initiation of each nutrient enrichment bioassay (no zooplankton information is available for early season periods, generally April and May). The zooplankton community N:P ratio ($N:P_z$) on a given date was calculated as a biomass-weighted mean:

$$N:P_z = \Sigma\,(N:P_i \times dw_i)/\Sigma\,dw_i$$

where $N:P_i$ is the N:P ratio of species i and dw_i is the dry mass concentration of species i on the sample date. Values of $N:P_i$ for *D. rosea*, *D. novamexicanus*, and *D. thomasi* were determined in this study; values from Andersen and Hessen (1991) were used for *H. gibberum* and *Bosmina longirostris* (*H. gibberum*: 8.6:1, by mass; *B. longispina*: 11.9:1, by mass).

Results

In situ monitoring

The timing of ice-out and the establishment of thermal stratification varied somewhat during the study period, primarily as a result of an early ice-out in 1990. The relatively warm, dry winters that contributed to drought in northern California in recent years led to a relatively early ice-out date in 1990 relative to 1991 and 1992 (8 April vs. approximately 15 May). In 1990, Schmidt stability (S) increased steadily for 5 weeks when a sustained series of cold spring storms passed through the region, cooling the surface waters and nearly mixing the lake again. In 1991 and 1992, S increased more or less continuously in the weeks following ice-out and the lake achieved its maximum S on

Table 1. Summary of statistical analyses (ANOVA on final chlorophyll concentration) of nutrient enrichment experiments. Statistical significance of main and interaction effects is indicated. n.s. = nonsignificant ($p > 0.05$), $*p < 0.05$. A dash indicates an effect that could not be, or was not, estimated due to insufficient replication, lack of treatments, or other problems.

	Experiment										
	1	2	3	4	5	6	7	8	9	10	11
1990	19 April	25 April	2 May	10 May	2 June	9 June	11 July	2 Aug	22 Aug	16 Sept	
P	*	*	*	*	*	n.s.	*	*	n.s.	*	
NH_4	*	n.s.	*	*	*	*	n.s.	*	n.s.	*	
NO_3	n.s.	n.s.	n.s.	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	
P×NH_4	*	n.s.	*	*	*	n.s.	n.s.	*	—	*	
P×NO_3	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	—	n.s.	
NH_4×NO_3	*	n.s.	n.s.	n.s.	*	*	n.s.	*	n.s.	n.s.	
P×NH_4×NO_3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	—	n.s.	
1991	17 May	22 May	2 June	10 June	30 June	21 July	9 Aug	13 Sept			
P	*	*	*	*	*	*	*	*			
NH_4	*	*	*	*	*	*	*	n.s.			
NO_3	*	n.s.	*	*	*	—	—	n.s.			
P×NH_4	*	n.s.	*	*	*	*	*	n.s.			
P×NO_3	n.s.	n.s.	*	*	*	—	—	n.s.			
NH_4×NO_3	n.s.	n.s.	*	*	n.s.	—	—	n.s.			
P×NH_4×NO_3	*	n.s.	*	*	n.s.	—	—	n.s.			
1992	11 May	16 May	23 May	30 May	7 June	19 June	26 June	10 July	31 July	21 Aug	10 Sept
P	*	*	*	*	*	*	*	*	*	*	*
NH_4	n.s.	n.s.	n.s.	*	*	*	*	*	*	*	*
NH_4×P	n.s.	n.s.	n.s.	*	*	*	*	*	*	*	*

approximately the same date in all years. The decrease in *S* during fall cooling began on approximately the same date in all years but occurred somewhat more rapidly in 1990 than in 1991 and 1992.

The dynamics of NO_3-N and NH_4-N in Castle Lake also varied during this period. In 1990 and especially 1992, NO_3-N and NH_4-N were high (>40 μg/L) in the surface layers under the ice immediately prior to ice-out (Fig. 1). However, in 1991, NH_4-N and especially NO_3-N in the surface waters at ice-out were less than 50% of their 1990 and 1992 values (NH_4-N: approximately 20 μg/L; NO_3-N: approximately 10 μg/L). In all years, initial inorganic N pools were rapidly depleted during the weeks following ice-out. In 1990, NO_3-N concentrations were largely depleted within 30–40 d; there was no evidence of an impact of the early season partial water column destabilization on NO_3-N and NH_4-N concentrations. In 1991, depletion of lower initial NO_3-N pools was more rapid and appeared complete (i.e., concentrations near the analytical detection limit) within 20 d following ice-out. The rate of depletion of NH_4-N concentrations from high spring values to values typical of summer conditions was similar (<1 month) in 1990 and 1991. On the other hand, NO_3-N and NH_4-N appeared to decrease more slowly in 1992; concentrations near the detection limit were not reached until 60–70 d after ice-out.

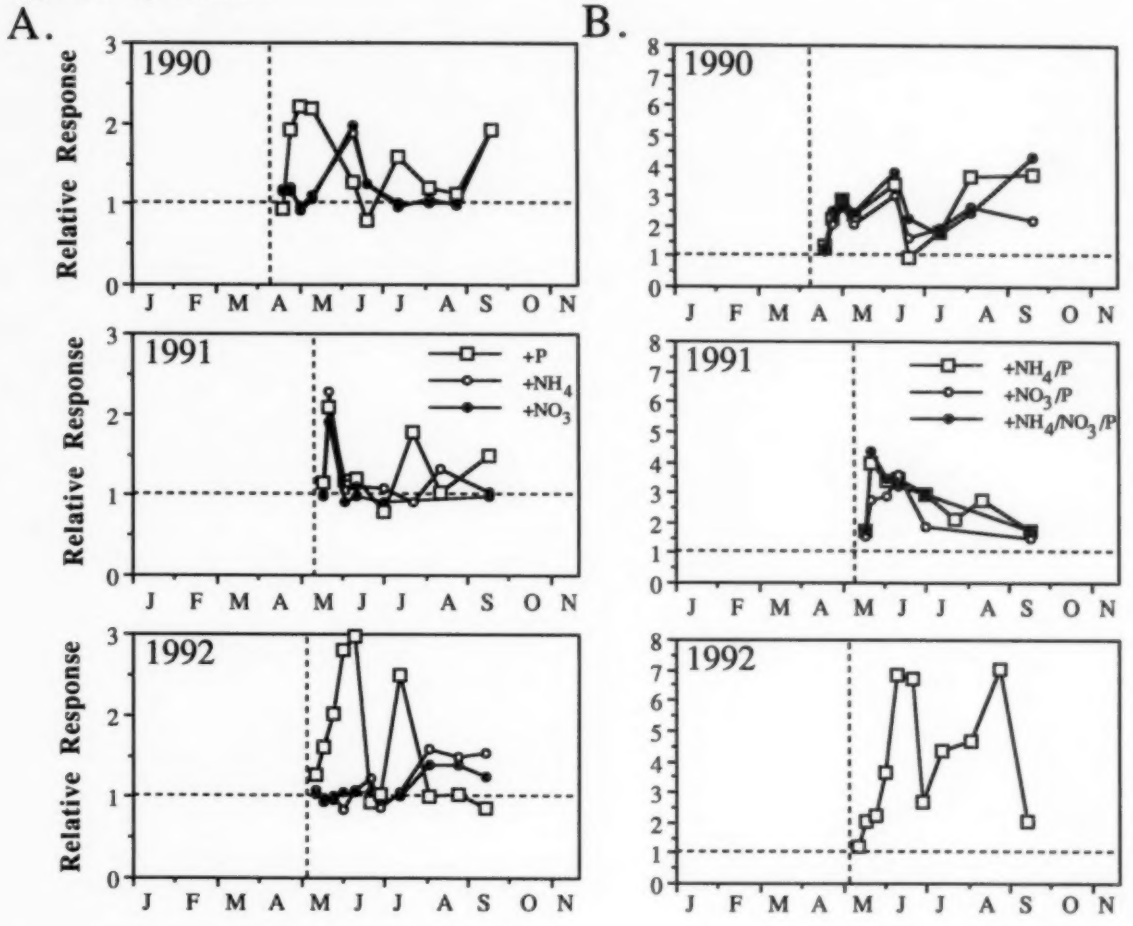
The dynamics of SRP in the Castle Lake surface waters (Fig. 2A) were considerably more erratic than for inorganic N. In 1990, SRP was low under the ice, increased following ice-out, and then varied erratically (0.5–4.5 μg/L) for the rest of the study period. In 1991, SRP appeared to decrease immediately prior to ice-out, increased during the first weeks of the ice-free period, and then varied very erratically (below detection (approximately 0.4 μg/L) to 5.5 μg/L) over the rest of the sampling season. The early season dynamics of SRP in 1992 resembled those of 1991 but in 1992, SRP declined during the sampling period.

Because changes in inorganic N were large relative to the smaller and less systematic variations in SRP, temporal changes in the N:P ratio of inorganic nutrient pools largely reflect the dynamics of dissolved inorganic N (Fig. 2B). In 1990 and 1992, the N:P ratio was high (>30:1, by mass; >65:1, by atoms) under the ice but declined dramatically following ice-out and varied between 2:1 and 7:1 (by mass; 4.9–15:1, by atoms) throughout the rest of the period. In contrast, the under-ice inorganic N:P ratio in 1991 was considerably lower (approximately 15:1, by mass; 33:1, by atoms), declined following ice-out, and varied between 0 (both NH_4-N and NO_3-N below detection) and 17:1 (by mass; 37:1, by atoms) for the rest of the season.

Nutrient limitation experiments

Overall ANOVA's of final chlorophyll concentration in nutrient bioassays were statistically significant ($p < 0.05$) for all 29 experiments (Table 1). Both N and P appeared to be important as potential growth limiting factors in Castle

Fig. 3. Results of nutrient enrichment bioassays performed during 1990–1992. (A) Relative growth responses (mean treatment chlorophyll / mean control chlorophyll) to single nutrient enrichments; (B) relative growth responses to combined N + P enrichments. Vertical broken lines indicate the timing of ice-out in each year. Note change of scale for growth response for Fig. 3A and 3B.



Lake, and there were consistent synergistic interactions in their effect on algal growth in these experiments. The effect of P was statistically significant as a main effect in 28 of the 29 experiments completed, while N (as NH_4-N or NO_3-N) was significant in 25 of 29 experiments (Table 1). Statistically significant ($p < 0.05$) N × P interaction terms occurred in 21 of the 28 experiments where they could be assessed (Table 1). Phytoplankton responded similarly to N added as NH_4 or NO_3 (Fig. 3); the relative magnitude of phytoplankton response to NH_4-N addition in an experiment correlated strongly with the relative response to NO_3-N ($p < 0.05$, $r^2 = 0.87$) with a slope (1.07) not significantly different from 1. However, NO_3 was statistically significant as a main effect in only 7 of 16 experiments (6 experiments, $p < 0.05$; 1 experiment, $p < 0.10$), while NH_4 was significant ($p < 0.05$) in 16 of 18 experiments (Table 1).

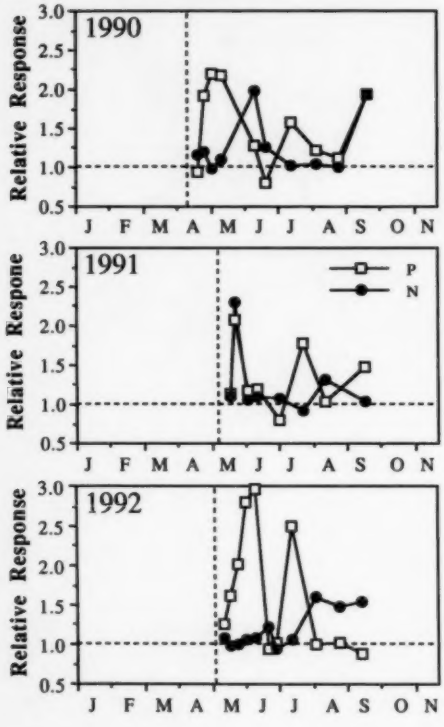
While overall ANOVA models were always statistically significant, the magnitude of growth response to various treatments and treatment combinations varied considerably both inter- and intra-annually (Fig. 3). The most obvious interannual difference in nutrient limitation involves the response of phytoplankton to single nutrient additions in 1991 versus those in 1990 and 1992 (Fig. 4). In 1991, responses to enrichment by N or P alone were generally weak and on only three occasions indicated strong limitation by a single nutrient (P in experiments 6 and 8, N in experiment 7). In contrast, in 1990 and 1992, experiments with strong response to single nutrient enrichment were common. In 1991, the mean maximum response to single nutrient enrichment was 1.44 (±0.15 SE), while in the 1990 and 1992, mean values were 1.66 (±0.15) and 1.82 (±0.20), respectively. Despite weak responses to single nutrient enrichment in 1991, responses to combined N + P enrichment were still substantial in that year as they were in 1990 and 1992 (Fig. 4).

Intraannual differences in springtime phytoplankton nutrient limitation were also observed (Figs. 3 and 4) and seemed to be associated with differences in early season

Table 2. Elemental composition of three common crustacean zooplankton species in Castle Lake. Values of % N and % P represent means (±SD) of triplicate determinations (80–150 animals per replicate) for each element. Samples were prepared on one occasion during late summer for each species.

Species	% N	% P	N:P (by mass)
Daphnia rosea	5.4 ± 1.5	1.15 ± 0.13	4.67
Diaptomus novamexicanus	6.9 ± 1.1	0.65 ± 0.06	10.61
Diacyclops thomasi	11.9 ± 4.6	0.95 ± 0.13	12.52

Fig. 4. Dynamics of potential growth limitation by N vs. P: relative growth response to P enrichment alone and N enrichment alone (maximum of the NH_4 and NO_3 treatment means). Vertical broken lines indicate the timing of ice-out in each year.



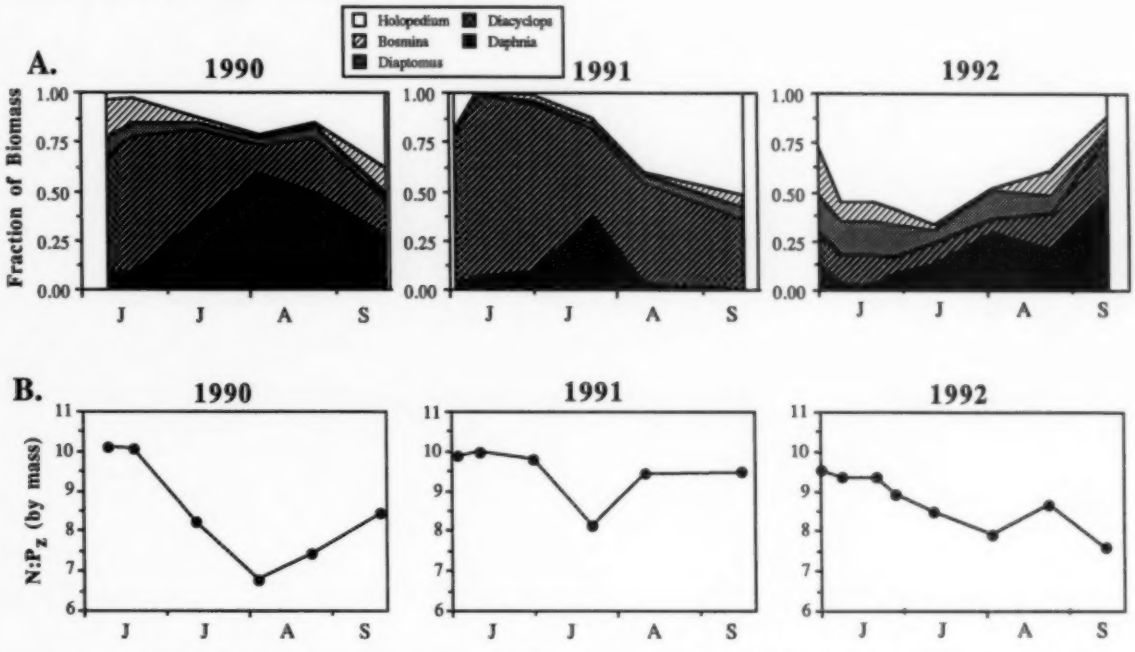
nutrient concentrations in the study years. Potential growth limitation by N or P was established early in all sampling years (Fig. 3). Statistically significant ANOVA results were obtained for the first experiment in all years (Table 1); these experiments were generally initiated within 1–4 d of ice-out. Initial peaks in growth response to N/P enrichment were achieved within 2–5 weeks following ice-out; this timing seemed to correspond with the timing of depletion of epilimnetic nutrient pools. In 1991, inorganic N was depleted most rapidly and peak enrichment response occurred at 2 weeks after ice-out, while in 1992, inorganic N was depleted more slowly and peak bioassay responses occurred at 5 weeks (Fig. 3).

Following the depletion of spring nutrient pools and the development of phytoplankton nutrient limitation, intraannual variation in bioassay responses was characterized by cyclic dynamics and strong alternation in growth responses to N vs. P (Fig. 4). Growth response to P was particularly periodic: in each year, a strong P-response maximum was observed within the first 15–30 d following ice-out with a second maximum around 65–90 d following ice-out. In both 1990 and 1991, a third maximum in P response occurred in the final experiment (125–160 days following ice-out). In general, when phytoplankton responded strongly to P, their response to N was weak, and vice versa (Fig. 4). Only two experiments appear to diverge from this pattern; both of these experiments were performed either very late (the final experiment in 1990) or early (experiment 2 in 1991) in the sampling season (Fig. 4).

Association with zooplankton dynamics

The elemental contents of the three zooplankton species we evaluated differed considerably (Table 2). Similar to the results of Andersen and Hessen (1991), *Daphnia* had the lowest N:P ratio of species studied. *Daphnia rosea*'s low body N:P ratio (4.7, by mass) reflected both a low body N content as well as high P content (Table 2). The cyclopoid copepod *D. thomasi* and the calanoid copepod *D. novamexicanus* had high and similar N:P ratios (10.6–12.5), reflecting a particularly high body N content in *D. thomasi* but a low body P content in *D. novamexicanus* (Table 2). As a result, changes in the relative abundance of these species in the zooplankton community in Castle Lake during the study period (Fig. 5A) were accompanied by shifts in community N:P ratio ($N:P_z$; Fig. 5B). To test whether the alternation in the identity of the primary limiting nutrient (Fig. 4) was associated with changes in the N:P ratio of the zooplankton, an index of relative N and P limitation was calculated for each experiment (N/P index = maximum response to single N enrichment in an experiment divided by the P response in that experiment) and compared with $N:P_z$ for the zooplankton community present on the sampling date closest to the initiation of each enrichment experiment. For 1990 and 1991, a significant ($p < 0.05$) positive correlation between the N/P index and $N:P_z$ was observed (Fig. 6A), indicating a tendency for phytoplankton to be most strongly limited by N when zooplankton community biomass was dominated by species with high body N:P ratios (i.e., *Diaptomus* and *Diacyclops*) but limited more strongly by P when *Daphnia* (with a low body N:P) was dominant. However, the 1992 N/P index and $N:P_z$ data did not fit the 1990–1991 relationship (Fig. 6B), and as a

Fig. 5. (A) Dynamics of the relative contribution of various crustacean zooplankton taxa to total crustacean zooplankton biomass during the summer sampling periods; (B) dynamics of the zooplankton community N:P ratio ($N:P_z$) during the summer sampling periods.



result, no correlation between these parameters existed for the entire 3-year data set.

The results of the zooplankton grazing experiment comparing the effects of *Daphnia* and *Diaptomus* on ambient concentrations of SRP and NH_4-N support the correlation in 1990 and 1991 between relative N vs. P limitation of phytoplankton and zooplankton N:P ratios: concentrations of SRP increased with increasing *Diaptomus* densities, while SRP *declined* in the presence of increasing *Daphnia* (Fig. 7A). Conversely, NH_4-N concentrations increased significantly in response to increasing *Daphnia* but declined somewhat (but nonsignificantly) with increasing *Diaptomus* (Fig. 7B). As a result, the inorganic N:P ratio increased dramatically with increasing *Daphnia* grazing but decreased significantly with enhanced *Diaptomus* (Fig. 7C). Thus, it appears that *Daphnia* (a low N:P species with high body P demands) differentially increases N availability relative to P and is likely to accentuate P limitation in phytoplankton. Conversely, *Diaptomus* (a high N:P species with relative low P demands) appears to differentially increase P availability relative to N and is likely to accentuate phytoplankton N limitation.

Discussion

Cullen (1991) discussed the implications of various nutrient bioassay procedures in evaluating the nature of phytoplankton nutrient limitation, distinguishing between studies that examine yields and those that evaluate processes (e.g., biomass vs. productivity), and delineating the role that time lags and grazer exclusion may play in complicating interpretation. Thus, the potential exists that deliberate macrograzer exclusion and inadvertent micrograzer damage in our experiments may have led to an overestimate of the strength of in situ nutrient limitation. As discussed by Cullen (1991), one can evaluate the importance of this influence by considering whether algae, freed from grazing control, grow considerably in unenriched controls. In our 1990 and 1992 experiments, control growth rates were generally less than 0.1/d, while growth rates in experimental treatments usually exceeded 0.3/d. However, in 1991 experiments, control growth rates commonly exceeded 0.1/d and often reached values greater than 0.2/d. This suggests that the regulatory importance of grazers, either macrozooplankton or microconsumers, was somewhat greater in 1991 than in 1990 and 1992 and that the decreased enrichment responses to single nutrient enrichment observed in 1991 relative to 1990 and 1992 may at least partially be the result of changes in overall grazing intensity.

Alteration of environmental conditions, especially incubating the phytoplankton at favorable light intensities, may also induce nutrient limitation in phytoplankton (Elser and Kimmel 1986) and thus artificially increase the magnitude of algal responses to nutrient enrichment. Thus, the potential for light limitation needs to be considered. We do not feel that this was important in Castle Lake during our study because Castle Lake is quite clear (depth of 1% light penetration: 17–23 m) and the epilimnion depth was usually 4–5 m during the study, with a maximum of 8–9 m prior to fall overturn. Finally, Cullen (1991) also discussed the possibility that time lags resulting from physiological processes (Lean and Pick 1981) or delays before community

Fig. 6. Relationship between the relative severity of N vs. P limitation (N/P index = relative response to N added singly/relative response to P added singly) and the zooplankton community N:P ratio ($N:P_z$). (A) Data for 1990–1991; (B) data for 1990–1992. High values of the N/P index indicate stronger growth limitation by N, while low values indicate P-limited phytoplankton growth.

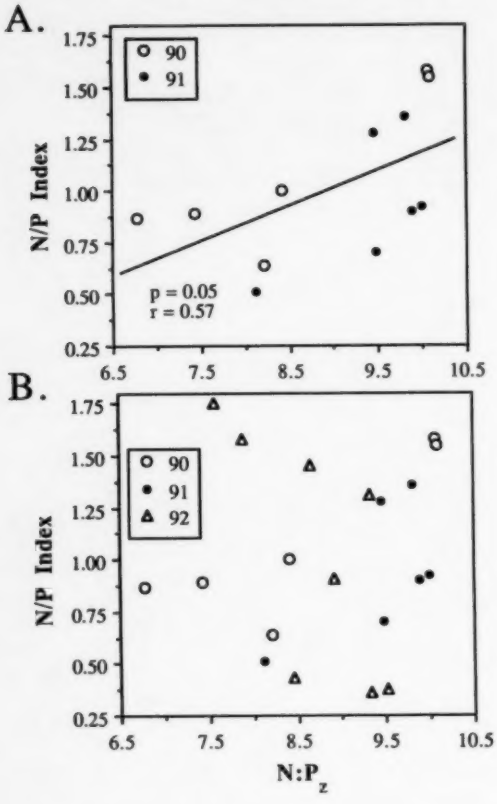
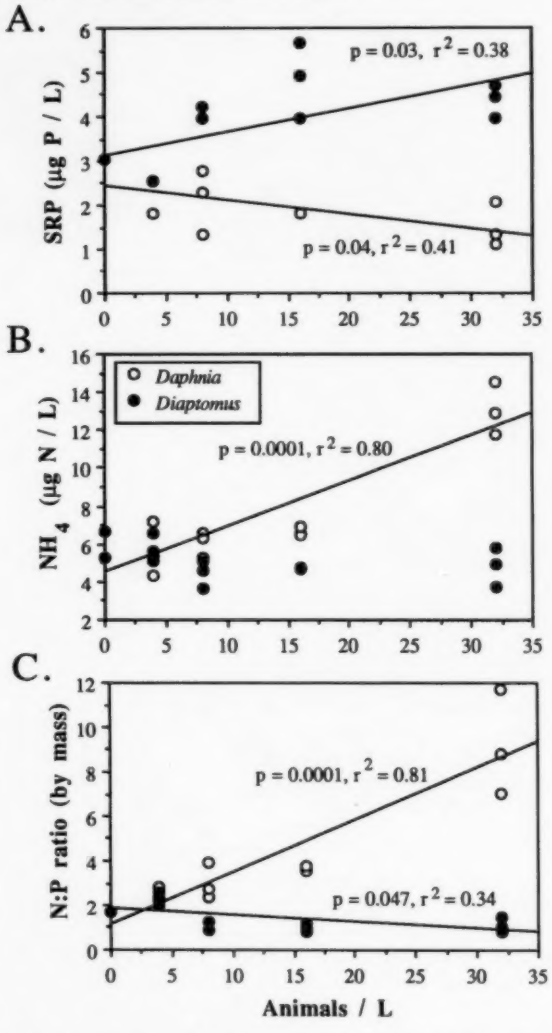


Fig. 7. Responses of inorganic nutrient concentrations to manipulations of *Daphnia* and *Diaptomus* abundance in a 6-d field experiment during summer 1992. (A) NH_4- N; (B) SRP; (C) inorganic N:P ratio (NH_4-N/SRP). NO_3-N was undetectable on this date.



subdominants respond to experimental treatments may complicate interpretation of enrichment bioassays. Since we have only beginning and end-point determinations of algal biomass, we cannot evaluate whether such time lags were important in our experiments. However, in a study of three oligotrophic Michigan lakes, close qualitative and quantitative correspondence was found between the results of nutrient enrichment bioassays and physiological indicators of N/P limitation (Elser et al. 1988), indicating that temporal dynamics of growth responses to enrichment can be primarily interpreted in terms of changes in nutrient availability and demand rather than changes in the operation of potential time lags in experiments such as these.

With these caveats in mind, the results of our enrichment experiments in Castle Lake indicate that *both* N and P are potentially important as short-term growth limiting factors in this system. N and P were simultaneously significant as main effects in 24 of 29 experiments (Table 1). The dual importance of N and P in limiting algal growth is also demonstrated by the frequency of significant N × P interaction terms (Table 1) and by the considerably larger growth responses that resulted from combined N and P addition relative to single addition of those elements (Fig. 3). Thus, the results of our intensive study of *intra*lake variation in the response of phytoplankton growth to N and P enrichments are similar to those obtained by Elser et al. (1990) in their examination of *inter*lake N/P limitation patterns. Elser et al. (1990), in a literature survey of short-term nutrient amendment studies of nutrient limitation in a variety of North American lakes, found that N amendment was just as likely to produce a significant growth response as was P amendment and that the magnitude of growth response was as large for N as for P. They also found that the frequency and magnitude of growth responses

to combined N and P enrichment were considerably greater than for addition of either element alone, a pattern that was commonplace in this study.

Interannual variation in patterns of nutrient limitation appeared to be primarily associated with differences in spring nutrient concentrations in 1991 relative to 1990 and 1992. In 1991 the weakest growth stimulation in response to single nutrient amendments was observed (Fig. 4) but strong synergistic effects of combined N + P addition were still observed (Fig. 3B). This reduction in phytoplankton growth response to single nutrient amendment coincided with considerably lower under-ice inorganic N concentrations in 1991 (approximately 28 μg dissolved inorganic N/L) than in 1990 and 1992 (approximately 75–90 μg dissolved inorganic N/L; Fig. 1), resulting in low spring inorganic N:P ratios (Fig. 2B). Low spring dissolved inorganic N concentrations indicating lower N availability in 1991 likely reduced the potential for phytoplankton to respond strongly to P enrichment, as growth would be rapidly constrained by available N after P is added. Thus, it appears that interannual shifts in bioassay responses in our study reflected variation in the accumulation of nutrients under the ice that produced high inorganic N:P ratios in 1990 and 1992 relative to 1991. In large, deep lakes, interannual variation in spring nutrients can be driven by variation in nutrient loading from deep-mixing processes (Goldman et al. 1989), while in shallower lakes with extensive watersheds, interannual nutrient loading shifts are largely driven by variation in runoff quantity due to weather differences and in nutrient concentrations due to watershed activities (Horne and Goldman 1994). Further study will be needed to understand the processes regulating nutrient concentrations present at ice-out both in Castle Lake and in similar lakes where spring nutrient inputs arise primarily due to under-ice processes and the brief period of nutrient loading during spring snowmelt from a relatively small watershed.

Intraannual patterns of variation in nutrient limitation were perhaps more striking than the differences we observed between years and appeared to be associated with biotic processes driven by consumer stoichiometry. While others have made observations of rapid shifts in the identity of the nutrient primarily limiting to phytoplankton (Lehman and Sandgren 1978; Elser et al. 1988), our consistent observation of a periodic variation in the severity of nutrient limitation (Fig. 4) is, to our knowledge, the first of its kind. As in the study of Elser et al. (1988), shifts in the identity of the primary limiting nutrient (N vs. P) were extremely rapid and frequently occurred in less than 2 weeks (Fig. 4). The correlation in 1990 and 1991 between the N/P index and the N:P ratio of zooplankton community ($N:P_z$) (Fig. 6A) and the strong differential effects of *Daphnia* and *Diaptomus* on inorganic N and P concentrations (Fig. 7) support the predictions of stoichiometric theory (Sterner et al. 1992) that elementally contrasting zooplankton species can qualitatively affect N and P availability in lakes. Thus, it appears that the periodic nature of intraannual variation in N/P limitation in this system may be associated with the summer dynamics of zooplankton species that differ in their body N:P ratios. However, this suggestion is tentative at this point given the considerable variation in relative N/P limitation remaining unexplained by $N:P_z$ in the 1990–1991 data (Fig. 6A) and the observation that alternation in N/P limitation in 1992 did not correlate with $N:P_z$ (Fig. 6B). We have no relevant data to help us account for the difference between 1992 and 1990–1991 with respect to the N/P index and $N:P_z$. We can point out that it is primarily the final three experiments of 1992 that do not conform with the 1990–1991 pattern; during this period in 1992, nutrient limitation shifted to N limitation (Fig. 4) despite a predominance of *Daphnia* (with a low body N:P ratio) in the zooplankton community (Fig. 5). In addition, we note that our observations in 1992 followed reinstatement of rainbow trout stocking in fall 1991 and thus it is possible the planktonic dynamics were in a state of flux following alterations in the planktivory regime.

Our study provides evidence that, similar to ecosystem processes that have received greater attention (e.g., primary productivity), patterns of nutrient limitation of phytoplankton growth exhibit considerable interannual and intraannual variation. Interannual variation appeared to result primarily from processes that produced strong differences in spring nutrient concentrations, apparently affecting patterns of nutrient limitation by enhancing simultaneous constraints on phytoplankton growth from both N and P (as demonstrated by weak enrichment responses to single N or P amendments) in 1991 when spring N concentrations were low. Intraannual variation in nutrient limitation appeared to be associated with biotic interactions, as evidenced by correlations in 1990 and 1991 between cyclic patterns of N/P limitation and the N:P ratio of the zooplankton assemblage. However, our evidence is equivocal on this point and further data are needed. Limnologists are now making progress in identifying and synthesizing the contributions of external factors (such as variable hydraulic loading and physical forcing) and internal, ecological interactions (such as grazing impacts and shifts in plankton size structure) affecting variation in lake primary productivity (Carpenter 1988; Jassby et al. 1990; Carpenter and Kitchell 1993). A further challenge will be to integrate this ongoing synthesis with patterns of interannual and intraannual variation in nutrient limitation (such as those indicated by the data presented here) and so achieve a fuller understanding of the factors regulating ecosystem productivity.

Acknowledgments

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Effect of juvenile steelhead (*Oncorhynchus mykiss*) on age-0 and age-1 brown trout (*Salmo trutta*) survival and growth in a sympatric nursery stream

John F. Kocik and William W. Taylor

Abstract: We determined that juvenile steelhead (*Oncorynchus mykiss*) had little effect on the survival and growth of age-0 and age-1 brown trout (*Salmo trutta*) in a Lake Huron tributary. Starting in 1989, we surveyed wild brown trout juveniles in two stream sections. Unfed steelhead fry were introduced into one section in May of 1990 and 1991, establishing moderate populations. Assessments continued through 1992. Steelhead had no measurable effect on juvenile brown trout abundance or survival. Steelhead did negatively influence age-0 brown trout growth, but this effect was minor compared with intraspecific interactions and abiotic factors. We attribute much of the brown trout success to their larger size. In June sampling, brown trout were typically 42% larger than steelhead, as would be common in the region. Variability in factors such as spawner–alevin interactions or climate could alter the timing of emergence or initial abundance, causing a more substantial effect on growth and, potentially, survival. Fishery managers need to evaluate these factors for the stocks in question before combining these species.

Résumé : Nous avons déterminé que le juvéniles de truite arc-en-ciel anadrome (*Oncorhynchus mykiss*) avaient peu d'effet sur la survie et la croissance de truites brunes (*Salmo trutta*) d'âge 0 et d'âge 1, dans un effluent du lac Huron. À partir de 1989, nous avons surveillé les juvéniles sauvages de truite brune dans deux tronçons du cours d'eau. Nous avons introduit dans un de ces tronçons, en mai 1990 et 1991, des alevins non nourris de truite arc-en- ciel, qui y ont établi des populations de densité modérée. Les évaluations se sont poursuivies jusqu'en 1992. La truite arc-en-ciel n'avait aucun effet mesurable sur l'abondance ni sur la survie des juvéniles de truite brune. Elle avait bien une influence négative sur la croissance des truites brunes d'âge 0, mais cette effet était minime comparé aux interactions intraspécifiques et aux facteurs abiotiques. Nous attribuons une bonne partie du succès de la truite brune à sa taille plus grande. Lors de l'échantillonnage de juin, les truites brunes étaient de façon typique d'une taille supérieure de 42 % à celle des truites arc-en-ciel, ce qui est commun dans la région. La variabilité de facteurs comme les interactions géniteurs–alevins ou le climat pourrait modifier la période d'émergence ou l'abondance initiale, ce qui aurait un effet plus important sur la croissance et peut-être sur la survie. Les gestionnaires des pêches doivent évaluer ces facteurs chez les stocks en question avant de combiner ces deux espèces.
[Traduit par la Rédaction]

Introduction

Habitat alteration, introduced species, and overfishing led to the demise of many salmonine populations indigenous to North American streams (Parsons 1973; Hearn 1987; Fausch 1988). As a result, many stream ecosystems now contain complexes consisting of remnant-indigenous, transplanted, and introduced species. Many of these salmonine stocks have similar ecological requirements that could conceivably foster interspecific competition (Hearn 1987; Fausch 1988). In particular, salmonine complexes that have not coevolved are especially prone to the negative effects of competition (Hearn 1987). Over the past century, Great Lakes tributaries have epitomized these conditions. Introduced salmonines predominate, having replaced indigenous stocks (Parsons 1973). Brown trout (*Salmo trutta*) and steelhead (*Oncorhynchus mykiss*) are among the most successful introductions, having established self-sustaining populations throughout the Great Lakes basin for over 100 years

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Fig. 1. Map of Gilchrist Creek, Montmorency County, Michigan, detailing geographic location and the placement of test and control areas.

(MacCrimmon and Marshall 1968; Biette et al. 1981). Despite similar success, these species exhibit distinct life-history patterns in the Great Lakes. Brown trout typically use streams for their entire life history and lake-run populations are rare (MacCrimmon and Marshall 1968; Scholl et al. 1984). Steelhead use the riverine environment for spawning and as juvenile nursery areas for 1–3 years before migrating to the lakes, where they mature (Biette et al. 1981; Seelbach 1987, 1993). As such, steelhead distribution in the Great Lakes overlaps with brown trout in many unimpounded coldwater tributaries. Despite the importance of both species for sportfishing in North America, interactions between these species have received little attention.

Recent investigations suggest that competition with steelhead may be detrimental to brown trout populations (Kruger et al. 1985; Ziegler 1988). In the early 1980's, Michigan anglers expressed concern that the Pere Marquette River's brown trout fishery had declined. Resultant investigations by Kruger et al. (1985) revealed an inverse relationship between brown trout and steelhead abundance. Additionally, during the period when steelhead and brown trout parr (<150 mm) reside in sympatry, brown trout grew below the state average (Laarman et al. 1981; Kruger et al. 1985). After the steelhead smolted, growth rates of brown trout accelerated to above the state average. Further, diet and habitat use of age-0 brown trout and steelhead under allopatry and sympatry were similar (Ziegler 1988). This evidence led Kruger et al. (1985) and Ziegler (1988) to hypothesize that juvenile steelhead might be adversely affecting brown trout abundance and growth.

The goal of our research was to determine the influence of age-0 and age-1 steelhead upon brown trout population dynamics and the extent and mode of competition. We hypothesized that juvenile steelhead negatively influenced juvenile brown trout by resource competition. To test this hypothesis, we introduced steelhead fry into a stream with an established brown trout population and quantitatively assessed their influence upon brown trout survival and growth.

Materials and methods

Study site

Gilchrist Creek is a second-order coldwater stream located in Montmorency County, Michigan (Fig. 1). We selected it based upon its abundant allopatric brown trout and isolation from lake-run fish due to downstream dams. Brown trout have been the predominant fish in the stream since at least the 1960's, comprising over 95% of the fish biomass (Kocik 1992). The only other fish species commonly encountered were mottled sculpin (*Cottus bairdi*) and northern redbelly dace (*Phoxinus eos*).

Gilchrist Creek flows northeast through forested land into the Thunder Bay River, a Lake Huron tributary. Soils in this region are primarily glacial sands, and deep aquifers maintain extremely stable temperature and flow regimes (Richards 1990). Monthly average temperatures ranged from 1.4°C in January to 17.1°C in July, and the highest monthly coefficient of variation was less than 1%. Extreme temperatures over the course of the study were −0.2 and 21.8°C. In the study areas, the creek averaged 7.6 m wide and discharge averaged 1.1 $m^3 \cdot s^{-1}$. Primary substrates are sand with long expanses of gravel.

Study design synopsis

We established two study sections in Gilchrist Creek, each containing three 100-m study reaches separated by 25-m buffer zones (Fig. 1). We collected salmonine population data triannually from each reach starting in June 1989. In spring of 1990 and 1991, we stocked steelhead fry in the downstream study section, creating a sympatric (test) treatment. The upstream section was not stocked and contained an allopatric wild brown trout population (control) throughout our experiment. We continued posttreatment evaluations through June 1992. We tested the effect of introduced steelhead upon the daily instantaneous rates of mortality and growth for brown trout. An analysis of variance model was used to compare pre- versus posttreatment means within sections and between-section means (test versus control) within years.

Steelhead introduction

To simulate natural steelhead colonization, we stocked steelhead swim-up fry in 1990 and 1991. We felt that it was both impractical and undesirable to impede fish movement to conduct this experiment, so we took advantage of steelhead behavior to isolate the experimental sections. Because steelhead fry dispersion is minimal and predominantly downstream (Wentworth and LaBar 1984; Hume and Parkinson 1987), stocking steelhead only in a 700-m area inclusive of the downstream section created allopatric and sympatric areas (Fig. 1).

We targeted a density of 3500 age-0 steelhead·ha^{-1} in autumn based upon documented autumn densities in nearby streams of comparable habitat quality and productivity (Carl 1983; Seelbach 1993). Using Hume and Parkinson's (1987) fry stocking data for steelhead and other salmonids, we calculated 1990 stocking rates using a conservative survival rate of 10% in 1990 (35 534 fry·ha^{-1}). In 1991, we adjusted stocking densities using the lowest estimated survival rate (1.4%) to calculate stocking densities of 242 415 fry·ha^{-1}.

Eggs from wild Little Manistee River steelhead were reared at the Michigan Department of Natural Resources (MDNR) Wolf Lake Hatchery. We transported unfed swim-up fry to the test section and scatter-stocked them equally into 100-m^2 stream units (Wentworth and LaBar 1984). On 30 May 1990, we stocked steelhead fry averaging 25.5 mm (±0.199 SE) and 0.122 g (±0.003 SE) at 355 fry per unit. In 1991, we stocked fry averaging 27.1 mm (±0.212 SE) and 0.143 g (±0.004 SE) at 2424 fry per unit on 23 May.

Salmonine population assessments

We collected population data triannually from June 1989 to 1992 using the same protocol before and after steelhead introduction. Mark–recapture population assessments were made during postsalmonine emergence (June), the post-growing season (October), and later winter (February). We sampled each reach using a 250-V direct-current electrofishing tote barge. Salmonines collected on the single-pass marking run were measured for total length (to 1 mm) and weight (to 0.1 g) and marked with a reach-specific partial fin clip. After a 24-h redistribution period, we resampled each stream reach with a single-pass recapture run, recording the number of marked (total length only) and unmarked fish (total length and weight). Overall electrofishing efficiency was good, averaging 48%. Analysis of fin clip data indicated that trout movement between reaches was minimal (<1%). We used Chapman's adjusted Petersen method to estimate population abundance (Ricker 1975). Fish were aged using length–frequency analysis and scale reading validation, allowing separation of fish into cohorts (MacDonald 1987).

Brown trout and steelhead daily instantaneous mortality and growth rates were calculated separately by cohort and stream reach. The rates represent the period between point samples: summer – June to October; winter – October to February; spring – February to June; and poststocking-May to June for steelhead. We used salmonine density at each sampling to calculate daily instantaneous mortality rates (Z) by reach and species for each period (Ricker 1975):

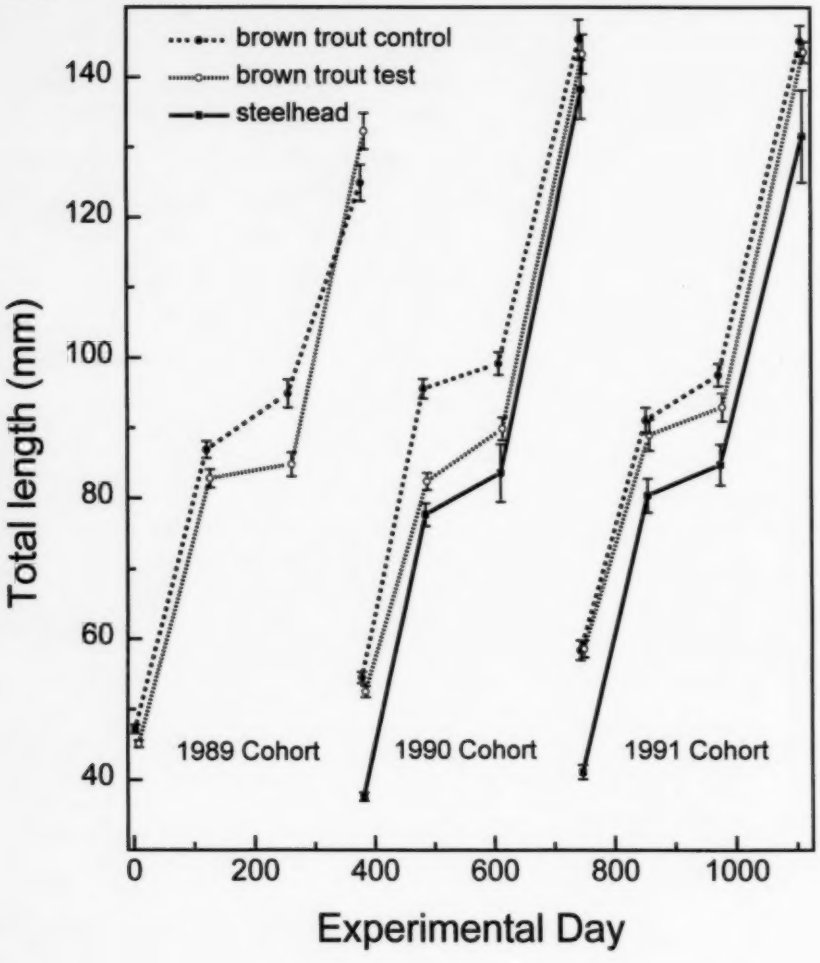
$$Z = \frac{\ln(\hat{N}_{t2}) - \ln(\hat{N}_{t1})}{(t_2 - t_1)}$$

where $\hat{N}_{t1}$ and $\hat{N}_{t2}$ = estimated number of fish at time 1 (t_1) and time 2 (t_2) and time units are days.

To assess growth, we calculated daily instantaneous growth rates (G) for each period (Ricker 1975):

$$G = \frac{\ln(\mathrm{TL}_{t2}) - \ln(\mathrm{TL}_{t1})}{(t_2 - t_1)}$$

Fig. 2. Comparison of the mean total length of 1989–1991 brown trout cohorts and 1990–1991 steelhead cohorts throughout their first 14 months, illustrating the differences in total length and growth between sections, species, and cohorts. Error bars represent 2 SE.



where TL_{t1} and TL_{t2} = mean total length (millimetres) at time 1 (t_1) and time 2 (t_2) and time units are days.

Statistical assessment methods

Analyses of population parameters were conducted by cohort. To clarify cohorts and relate changes to meaningful life-history periods, we assigned all salmonines a birth date of 15 April, corresponding to the typical date of brown trout emergence from redds. As such, age-0 fish were sampled in June, October, and February of their first year. All statistical comparisons used a critical level of ($\alpha = 0.05$).

We partitioned statistical analyses by date for point estimates (density and size) and by season for rate variables (Z and G). To account for nonnormality of rate variables, we used the RT-1 transformation to standardize these data (Conover and Iman 1981). We compared treatment differences in brown trout using analysis of variance (ANOVA) and single degree-of-freedom linear contrasts (Ott 1988). We designed contrasts to test biologically meaningful hypotheses: between-section differences within years (e.g., 1990: test versus control) and between-treatment differences within sections (e.g., test section: presteelhead versus poststeelhead). We used the Bonferroni routine ($\alpha = 0.05$) to adjust for the nonorthogonal designed contrasts, establishing an operative α of 0.01 (Ott 1988).

To further discern the influence of steelhead on brown trout, we constructed a multiple regression model to examine the relationship between brown trout total length (Y) and density of their corresponding brown trout ($X1$) and steelhead ($X2$) cohorts. We constructed separate models for age-0 and age-1 brown trout and steelhead in the downstream study section.

Table 1. Average number of brown trout·ha^{-1} and standard error (SE) in control and test sections ("s" denotes presence of sympatric steelhead cohort) of Gilchrist Creek for 1989–1991 cohorts assessed from age 0 and for 1988 cohort assessed from age 1.

		1988 cohort		1989 cohort		1990 cohort		1991 cohort	
Month	Age	Control	Test	Control	Test	Control	Test^s	Control	Test^s
June	0+			4640	9074	4101	5614	6465	4817
				(1601)	(4753)	(1196)	(1038)	(1220)	(1869)
October	0+			2002	1988	1907	3096	2460	1804
				(349)	(325)	(267)	(657)	(499)	(417)
February	0+			1640	2878	1189	1634	1651	1102
				(221)	(319)	(234)	(333)	(265)	(82)
June	1+	3545	836	1487	1283	991	656	1465	823
		(1322)	(231)	(248)	(132)	(102)	(161)	(340)	(65)
October	1+	1200	575	987	773	912	557		
		(154)	(51)	(40)	(100)	(185)	(108)		
February	1+	850	160	753	450	857	627		
		(179)	(54)	(157)	(53)	(106)	(184)		

Results

Steelhead introduction and population assessment

Stocking of steelhead swim-up fry established populations in the test section of Gilchrist Creek in both years. Daily instantaneous mortality from stocking to June was higher ($P = 0.02$) in 1991 (0.1395) than in 1990 (0.0850). Subsequently, mortality patterns and densities were similar for both cohorts ($P > 0.05$). Steelhead densities averaged 4603, 1769, and 355 fish·ha^{-1} in their first June, October, and February and 351 fish·ha^{-1} in their second June. At stocking, fry were larger in 1991 (27.1 ± 0.21 mm) than in 1990 (25.5 ± 0.20 mm), and the difference remained significant in June. However, higher summer G in 1990 (0.0070) compared with 1991 (0.0055) offset initial length differences, resulting in no significant difference in total length ($P = 0.49$) or weight ($P = 0.49$) by October. From that point onward, both cohorts grew similarly ($P > 0.05$; Fig. 2).

Brown trout abundance and mortality

Age-0 brown trout density varied from 4817·ha^{-1} in 1991 to 9074·ha^{-1} in 1989 in the test section and from 4101·ha^{-1} in 1990 to 6465·ha^{-1} in 1989 in the control section (Table 1). Brown trout abundance was not significantly different in any between-section or between-treatment comparisons of June or October samples. In February 1990, brown trout density in the test section (2878 fish·ha^{-1}) was significantly higher than in the control section ($P = 0.0051$). The higher February abundance indices in 1990 also contributed to significantly higher pretreatment densities in the test section ($P = 0.0004$). The posttreatment densities were lower, averaging 1634 and 1102 fish·ha^{-1} for 1990 and 1991 cohorts, respectively. Age-1 brown trout densities were not significantly different ($P > 0.05$) for any between-section or between-treatment comparisons (Table 1).

Summer Z was not significantly different for any contrast (Table 2). Age-0 winter Z was significantly lower ($P = 0.0029$) during the pretreatment assessment than after the introduction of steelhead in the test section. However, a high percentage of fish collected in February 1990 lacked reach-specific fin clips from the previous autumn, indicating movement into the test section from areas outside the study reaches. This age-0 influx in the test section was apparent only during the winter of 1989–1990. Spring Z was significantly higher in the test section than in the control section for both 1989 ($P = 0.0015$) and 1990 ($P = 0.0021$) cohorts. However, there were no significant differences in comparisons between pre- and posttreatment periods for the control section ($P = 0.6022$) or test section ($P = 0.3398$).

For age-1 fish, summer and spring Z were not significantly different ($P > 0.05$) for any between-section contrasts. The only Z comparison that was significant was the test section pre- versus posttreatment comparison ($P = 0.0063$). This difference was driven by the low Z rate (0.0004) of the 1990 cohort in the winter of 1991–1992. The result was lower Z in sympatry with age-0 steelhead in the test section, indicating an insignificant effect on age-1 brown trout abundance.

Brown trout size and growth

The size and growth of age-0 brown trout were variable throughout the course of this study (Fig. 2). Because total length and weight were highly correlated ($r^2 = 0.97$) and statistical comparisons of size yielded identical results using either variable, only length data are presented. In June, between-section comparisons within years showed no significant difference ($P > 0.05$) in size of age-0 brown trout. However, significant size differences existed in some February (1990, 1991) and October (1990) comparisons, where age-0 brown trout were larger in the control section than in the test section ($P < 0.05$). We also noted a trend of increasing size for age-0 brown trout each year of the experiment (Fig. 2).

In between-section comparisons, age-1 brown trout in the test section were significantly larger only in October 1989

Table 2. Seasonal daily instantaneous mortality rates (Z) of brown trout cohorts in control and test sections of Gilchrist Creek by cohort and average Z for presteelhead and poststeelhead periods by section. Summer represents June–October; winter represents October–February; spring represents February–June. An asterisk indicates significant differences at the 0.05 level for between-section or between-treatment comparisons.

		Allopatric cohorts				Test section sympatric				Sectional averages by treatment			
		1988 cohort		1989 cohort		1990 cohort		1991 cohort		Control sections		Test sections	
Season	Age	Control	Test	Control	Test	Control	Test	Control	Test	Pre	Post	Pre	Post
Summer	0+			0.0062	0.0107	0.0067	0.0058	0.0089	0.0081	0.0062	0.0078	0.0107	0.0048
Winter	0+			0.0013	−0.0028	0.0039	0.0050	0.0032	0.0037	0.0013	0.0060	−0.0028	0.0030*
Spring	0+			0.0009	0.0069*	0.0012	0.0069*	0.0010	0.0022	0.0009	0.0022	0.0069	0.0046
Summer	1+	−0.0081	0.0026	0.0037	0.0047	0.0010	0.0014			−0.0022	0.0010	0.0037	0.0014
Winter	1+	0.0028	0.0107	0.0025	0.0041	0.0003	0.0004			0.0027	0.0003	0.0074	0.0004*
Spring	1+	0.0071	0.0018	0.0026	0.0045	0.0037	0.0037			0.0049	0.0037	0.0032	0.0037

($P = 0.0074$) and June 1990 ($P = 0.0005$). All other between-section comparisons were nonsignificant. Pretreatment versus posttreatment differences were significant in all control and test section comparisons except the February test section comparison. In all cases, age-1 brown trout were larger posttreatment than pretreatment, indicating no negative effect of steelhead on age-1 size.

Daily instantaneous growth rate comparisons provide a more discernible examination of growth effects, since the confounding effect of initial size is reduced (Table 3). Between-section comparisons were significant only for the 1990 cohort in summer and spring. Age-0 summer G was significantly lower ($P = 0.0009$) in the test section (0.0043) than in the control section (0.0054) for this cohort Overwinter, the relative magnitude of G shifted, although the difference was not significant. By the next spring, G in the test section (0.0035) was significantly higher ($P = 0.0062$) than in the control section (0.0028). Between-treatment comparisons were significant, revealing lower average summer growth rates for age-0 brown trout ($P = 0.0012$) in sympatry. The same comparison in the control section revealed no such effect ($P = 0.1291$). Once brown trout reached age 1, no significant differences in G were found in between-section or between-treatment comparisons.

Multiple regression models

We developed multiple linear regression models for age-0 and age-1 brown trout to determine how the density of corresponding cohorts of brown trout and steelhead affects their size. The model for age-0 brown trout was significant ($P = 0.003$) and accounted for 38% of the observed size variation. The size of age-0 brown trout was inversely related to the number of age-0 steelhead and brown trout ($P < 0.01$). The standard partial regression coefficients for steelhead abundance (−0.476) were higher than those for brown trout abundance (−0.457), indicating that the influence of steelhead was more important.

The model for age-1 brown trout was also significant ($P < 0.001$), accounting for 46% of size variation and indicating an inverse relationship between size and density. Only age-1 brown trout abundance (−0.677) was a significant ($P < 0.01$) contributor to the model, suggesting that intraspecific competition influenced size more than interspecific competition by age 1.

Discussion

Salmonine abundance and mortality

Mean autumn densities of age-0 and age-1 steelhead were within the low end of ranges reported by Seelbach (1993). Daily instantaneous mortality rates for steelhead were highest between stocking and the June assessments. High initial mortality has also been observed in other steelhead fry stocking experiments (Wentworth and LaBar 1984; Hume and Parkinson 1987; Close and Anderson 1992). Despite a 700% higher stocking level in 1991, steelhead had equilibrated to comparable densities by June for both 1990 and 1991 cohorts. This similarity in densities between years suggests that we reached the stream's carrying capacity for steelhead. Close and Anderson (1992) provided the only other documented assessment of steelhead fry stocking

in the Great Lakes region. Their fry stocking project in Lake Superior tributaries yielded higher populations, with late summer densities averaging 19 250 fish·ha^{-1}. A major difference between the two introductions was that the streams stocked by Close and Anderson (1992) contained few resident salmonines. Gilchrist Creek contains an abundant brown trout population with average autumn densities of 3389 age-0, 863 age-1, and 197 age-2+ fish·ha^{-1}. Researchers have attributed much of the success of fry stocking to favorable flow conditions and minimal interactions with wild fish (Wentworth and LaBar 1984; Hume and Parkinson 1987; Close and Anderson 1992). Gilchrist Creek exhibited stable flows in the springs of 1990 and 1991, exempting discharge as an important mortality component. We suspect that interactions with juvenile brown trout (competition) and larger brown trout (predation) were major causes of steelhead mortality in this study (Alexander 1977). Our results suggest that the presence of established brown trout was a significant impediment to the establishment of a larger steelhead population.

The effects of steelhead on age-0 and age-1 brown trout survival were negligible. The only significant population-level response was lower age-0 brown trout abundance in the test section during winter following steelhead introduction. We observed several unmarked fish in the test area during the 1989–1990 winter, suggesting an immigration of age-0 brown trout from other stream reaches. Overwinter aggregations of brown trout occur as colder temperatures trigger reductions in territorial behavior and dominance hierarchies (Cunjak and Power 1986; Hearn 1987). Temperatures during the 1990 winter sampling were the coldest encountered, with daily air temperatures of −15°C compared with more seasonal temperature of 1°C. It is probable that these severe conditions caused brown trout to aggregate. Alternatively, because no immigration was apparent after steelhead introduction, this could be interpreted as evidence of competition for wintering habitat. However, combined brown trout and steelhead abundance in this section was lower in both posttreatment winters than brown trout abundance was in 1990. Given the lower total abundance, we feel that steelhead were not a deterrent to brown trout movements and that the cold temperature during sampling in February 1990 prompted immigration to these regions of significant groundwater discharge and plentiful overhead cover (Kocik 1992).

Steelhead and brown trout size and growth

Average size of brown trout and steelhead compared favorably with those from other studies in the Great Lakes. In this study, average age-0 (79 mm) and age-1 (164 mm) steelhead total length in autumn was in the upper size ranges (50–80 and 112–196 mm) reported by Seelbach (1993) for the Great Lakes region. Brown trout were also large compared with populations found elsewhere in Michigan, with autumn mean sizes of age-0 (89 mm) and age-1 (163 mm) brown trout above the state averages reported by Laarman et al. (1981). While steelhead sizes were comparable between years, the size of brown trout differed between years. These differences were not attributable to steelhead and followed similar trends in both test and control sections. Evidently, factors other than the presence

Table 3. Seasonal daily instantaneous growth rates (G) of brown trout cohorts in control and test sections of Gilchrist Creek by cohort and average G for presteelhead and poststeelhead periods by section. Summer represents June–October; winter represents October–February; spring represents February–June. An asterisk indicates significant differences at the 0.05 level for between-section or between-treatment comparisons.

		Allopatric cohorts				Test section sympatric				Sectional averages by treatment			
		1988 cohort		1989 cohort		1990 cohort		1991 cohort		Control sections		Test sections	
Season	Age	Control	Test	Control	Test	Control	Test	Control	Test	Pre	Post	Pre	Post
Summer	0+			0.0051	0.0051	0.0054	0.0043*	0.0041	0.0039	0.0051	0.0048	0.0051	0.0043*
Winter	0+			0.0006	0.0002	0.0003	0.0007	0.0006	0.0004	0.0006	0.0005	0.0002	0.0007
Spring	0+			0.0033	0.0037	0.0028	0.0035*	0.0026	0.0028	0.0033	0.0027*	0.0037	0.0035
Summer	1+	0.0018	0.0019	0.0012	0.0016	0.0015	0.0013			0.0015	0.0015	0.0018	0.0013
Winter	1+	0.0004	0.0004	0.0005	0.0004	0.0004	0.0003			0.0005	0.0004	0.0004	0.0003
Spring	1+	0.0027	0.0021	0.0020	0.0027	0.0012	0.0019			0.0024	0.0012	0.0024	0.0019

of steelhead, such as recruitment variability, intraspecific population dynamics, or abiotic factors, had a greater effect on brown trout size (Elliott 1984*a*, 1984*b*; Newman and Waters 1989). We believe that these factors controlled the observed year-to-year size differences.

Despite the apparently minor role of steelhead in influencing the overall size of brown trout, an effect on growth rate was found. Tests of treatment effects on summer growth were highly significant in the test section and not in the control section. The multiple regression model for age-0 brown trout also indicated a negative effect of steelhead densities on growth rates. The slower growth of brown trout in sympatry with steelhead suggests that steelhead interaction may inhibit brown trout growth. Rose (1986) found that steelhead inhibited brook trout growth in a Lake Superior tributary to the extent that their wintering mortality was increased. He observed this effect despite an initial sixfold weight advantage of brook trout. In our study, brown trout were only 1.4 times heavier than steelhead, but the effect of steelhead was not as great. However, steelhead were 50% more abundant than brook trout by the end of August in Rose's (1986) study, while in Gilchrist Creek population assessments, brown trout predominated. We theorize that the relative population densities of steelhead and brown trout influence interspecific competition between these species by controlling the extent of overlapping juvenile sizes and the abundance of food. The results of both studies indicate that, despite a size disadvantage, steelhead influenced the growth of larger salmonine species of the same cohort. This effect may be particularly important in years when other factors, both biotic and abiotic, have caused smaller brown trout size and slower growth.

A conceptual model of brown trout and steelhead interactions

To better assess our results in the context of present knowledge of salmonine life-history strategies and to formulate new hypotheses on the interactions between these species, we developed a conceptual model of the temporal life-history overlap of brown trout and steelhead. This model details the sympatric riverine life history of both species by categorizing periods of temporal overlap that could result in competition between the species.

Interactions between juvenile salmonids start at the postemergence stage and are influenced by the redd emergence gap between species. Our fry stocking regime simulated steelhead emergence in late May, a common time in the Great Lakes (Carl 1983; Rose 1986). Brown trout in Gilchrist Creek emerged in mid-April, also a common time in the region. While steelhead and brown trout are roughly the same size at hatch and emergence (Auer 1982), the emergence gap gave brown trout at least a 30-day head start in exogenous growth. While this gap simulated the regional norm, different genetic stocks or thermal regimes could alter this interval. In some Lake Michigan tributaries, brown trout emergence dates are as late as mid-May (Fausch and White 1986). Brown trout stocks that emerge later would likely lose the size advantage gained by early emergence.

The size spectra set up by the emergence gap may be important in determining the outcome of interactions in the postemergence stage, as both species utilize stream margins until midsummer (Sheppard and Johnson 1985; Baltz et al. 1991). In our study, age-0 brown trout were 40% larger than steelhead in June. Such a size advantage typically gives the larger salmonine an edge in competitive interactions (Chapman 1962; Fausch and White 1986; Hearn 1987). Antagonistic behavior at both an intraspecific and interspecific level starts shortly after emergence. Because steelhead are suspended in the water column and brown trout are more closely associated with the substrate (Kocik 1992), vertical stratification may allow habitat partitioning between these species. However, the shallow nature of marginal habitats likely negates this mechanism, resulting in direct interactions. Cunjak (1982) found that rainbow trout emergence appeared to cause a downward shift in the vertical position of brook trout, indicating interspecific interactions in similar habitats. Interactions between fish typically result in the formation of dominance hierarchies and territories (Fausch and White 1986; Hearn 1987). The fish that gain the most profitable positions are most likely to dominate, exhibiting superior growth and survival. The subordinate fish typically exhibit lower growth, higher mortality, and many emigrate from the area (Chapman 1962; Hearn 1987). We feel that the dominance of brown trout over postemergent steelhead resulted in high initial steelhead mortality or emigration. This observation is reinforced by the fact that a sevenfold increase in steelhead stocking rate failed to produce significantly more juveniles in the second year of introductions. As such, the deliberate introduction of steelhead fry into a stream with a healthy brown trout population had little effect on the brown trout population. Due to these early interactions, steelhead were unable to achieve sufficient densities to have a significant effect on brown trout through exploitive competition.

The summer growth stage is characterized by the habitat shift of age-0 fish to deeper and faster water. From this point onward, habitat use by the two cohorts is similar until steelhead outmigration (Raleigh et al. 1984, 1986; Ziegler 1988). In deeper water, vertical stratification may become a more effective habitat segregation mechanism (Raleigh et al. 1984, 1986; Sheppard and Johnson 1985; Baltz et al. 1991; Kocik 1992). Thus, the influence of segregation might be to reduce or eliminate potential competition, thereby increasing total salmonine abundance (Hearn 1987; Fausch 1988). The faster growth rate of steelhead allows them to reduce the size advantage of brown trout over the course of the growing season, often surpassing brown trout by their second summer (Kruger et al. 1985; Ziegler 1988). If steelhead abundance is high enough at this point, the influence of steelhead on brown trout growth and survival would likely increase.

The limited influence of steelhead on brown trout as juveniles suggests that if an important negative interaction exists, it must be occurring at another stage or be a result of cumulative effects. In the Great Lakes region, brown trout typically spawn from October to November and emerge from redds between March and May (MacCrimmon and Marshall 1968; Kocik 1992). Steelhead spawning starts in December and is most frequent from late February through April, with emergence occurring in May and early

June (Seelbach 1987, 1993). As such, steelhead redd superimposition could physically damage or prematurely displace brown trout from redds, decreasing survival from spawning to emergence (Hayes 1987). Spawning overlap could also foster streambed overseeding that could degrade the water quality of redd areas, adversely influencing alevin survival (Kocik and Taylor 1991). Both factors may be particularly acute given spawning habitat limitations and high spawner abundance common in Great Lakes tributaries (Kocik and Taylor 1987, 1991). Thus, superimposition and overseeding by steelhead could negatively affect brown trout abundance, altering initial postemergence ratios of brown trout to steelhead.

Annual variations in climate could also influence brown trout – steelhead interactions. The steelhead superimposition period and the emergence gap are particularly susceptible to environmental variability. Year-to-year variation in water temperature and discharge provides two scenarios for potential environmental influence upon interactions between these species. Natural temperature variation can cause up to a 50-day difference in mean brown trout emergence dates as well as influence size (Elliott 1984*a*). Steelhead, while affected by temperature differences, have a shorter incubation period, which occurs later in the spring when temperatures are typically warmer. This abiotically driven variation could reduce or eliminate a brown trout size advantage at the postemergence stage. In our study, the 1990 and 1991 brown trout cohorts were 42% larger than steelhead. However, if steelhead had been introduced in 1989, brown trout would have been only 17% larger. The timing of spring floods can also affect salmonine production (Kocik and Taylor 1987). If high discharge occurs during the brown trout – steelhead emergence gap, it is likely that swim-up brown trout would be adversely effected, while steelhead would be sheltered in the gravel. As such, differences in abiotic factors between years and between river systems could alter the growth and survival of these species differentially, influencing competitive interactions.

Conclusions

In this study, competition between juvenile brown trout and steelhead favored brown trout, with significant interactions occurring during the postemergence stage. After this stage, the species appear to effectively partition available habitat. Similarly, Hayes (1989) noted that lake-run rainbow trout and brown trout juveniles in New Zealand appear adapted to complementary niches and social roles. These studies indicate that the juveniles of these two species can coexist with adequate temporal segregation between emergence (Hayes 1989; Kocik 1992).

The history of natural reproduction and colonization of brown trout and steelhead in the Great Lakes indicates their success in these systems. However, recent evidence suggests that this balance may be shifting to favor steelhead (Kruger et al. 1985). Our investigation into juvenile interactions indicates that juvenile steelhead do not appear to be a cause of declining brown trout populations. Research on spawning habitat selection, stock-specific spawning dates, the role of variable environmental factors, and the role of cumulative effects is needed to assess these factors in the Great Lakes. The connection between tributaries and the Great Lakes is also important, especially considering the dramatic changes that have occurred due to anthropogenic activities. These changes in lacustrine ecosystems influence the relative advantages of migratory or resident life-history strategies (Gross 1987) and play an important role in the balance between brown trout and steelhead populations. Thus, we urge fishery managers to consider the life history and ecology of specific stocks and the influence of variable climatic and environmental factors when managing these species in sympatry.

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Abundance and distribution of pelagic 0-group cod (*Gadus morhua*) in Newfoundland waters: inshore versus offshore

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Abstract: The Northern cod stock has previously been described as undergoing a large-scale denatant–contranatant migration during which spawning occurs primarily in the north off Labrador. Eggs and larvae are advected southward to the inshore areas along the NE coast of Newfoundland where they settle as juveniles, then there is a gradual return migration to the northern spawning areas. We examined abundance distributions of pelagic juvenile (0-group) cod in the fall between inshore and offshore areas to determine if these fish occurred predominantly inshore following pelagic drift. Comparison between areas demonstrated no differences in abundances and mean sizes. Our results do not support the concept of large-scale advection of eggs and larvae spawned offshore to inshore areas, but indicate that cod eggs spawned offshore remain offshore. Back calculations of spawning times indicate the pelagic juvenile cod sampled offshore were spawned predominantly in April, whereas fish caught inshore were spawned predominantly in May–June, as well as April. Estimates of spawning abundance indicate that the inshore areas along the NE coast of Newfoundland may represent <10% of eggs spawned annually. Historically, the offshore area appears to have been the primary habitat for pelagic juvenile cod.

Résumé : On a déjà soutenu que le stock de morue du Nord connaît une migration à grande échelle avec phase passive puis déplacement à contre-courant, la ponte se passant surtout au nord du Labrador. Les oeufs et les larves étant amenés par advection vers le sud jusqu'aux eaux côtières du nord-est de Terre-Neuve, où ils s'installent au stade juvénile, puis il y a une migration graduelle de retour vers les frayères du nord. Nous avons examiné la répartition de l'abondance des morues juvéniles pélagiques (groupe 0) à l'automne entre les zones côtières et hauturières pour déterminer si ces poissons se retrouvaient principalement près des côtes à la suite de la dérive pélagique. La comparaison entre zones n'a révélé aucune différence dans l'abondance ni dans la taille moyenne. Nos résultats ne confirment donc pas l'idée d'une advection à grande échelle des oeufs et des larves éclos au large en direction des eaux côtières, mais indiquent au contraire que les oeufs de morue pondus au large y restent. Le calcul rétroactif des périodes de ponte indique que les morues juvéniles pélagiques prélevées au large avaient été pondues principalement en avril, tandis que les poissons capturés dans les eaux côtières avaient été pondus principalement en mai–juin, ainsi qu'en avril. Des estimations de l'abondance de la ponte indiquent que les zones côtières de la côte nord-est de Terre-Neuve pourraient représenter <10% des oeufs pondus chaque année. Historiquement, la zone du large semble constituer le principal habitat des morues juvéniles au stade pélagique.

[Traduit par la Rédaction]

Introduction

The Northern cod stock, the largest and most valuable cod stock in the Northwest Atlantic, has been managed as a single unit bounded by the Northwest Atlantic Fisheries Organization (NAFO) Divisions 2J3KL, stretching over 6 degrees of latitude from Hamilton Bank off southern Labrador to the northern part of the Grand Bank (Fig. 1). This stock has traditionally been regarded as a stock complex, and is composed of several spawning units associated with each of the offshore banks (Templeman 1981, 1989; Lear 1984). Formation of the Div. 2J3KL management unit was based on a description of the biology formulated through several early studies (Halliday and Pinhorn 1990). Central to this description was the understanding that these fish undergo a large denatant–contranatant migration (Templeman 1979, 1981; Lear and Green 1984). Spawning reportedly occurs primarily in deep water along the slope

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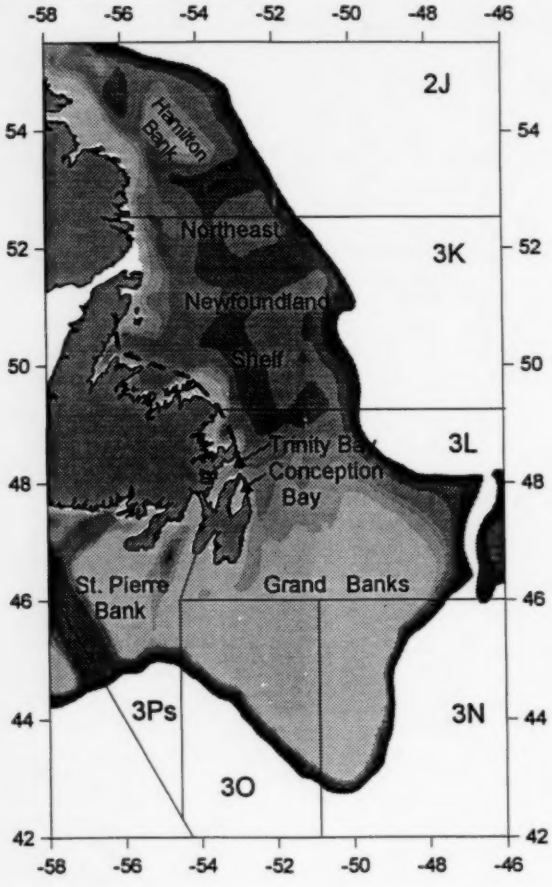
Fig. 1. Ocean area off Newfoundland and Labrador indicating the major banks as well as in the inshore bays referred to in this study. The inshore area referred to lies shoreward of the dashed line.



of the continental shelf off Labrador in March and April (Serebryakov 1965, 1967; Postolaky 1968; Templeman 1981). Following spawning, eggs and larvae develop slowly in cold surface waters for periods of 40–60 days and are thought to be carried south and shoreward 450–750 km by the Labrador Current to the large bays and numerous inlets of eastern Newfoundland where these fish develop and settle to the bottom in the fall (Templeman 1981; Lear and Green 1984). The inshore areas, therefore, appear to serve as the primary nursery areas for these young cod as they grow and develop.

Recent work indicates that the above description is too simplistic. Templeman (1989) concluded there was evidence for inshore spawning of cod, which occurred later than the offshore spawning components. Recently, Helbig et al. (1992) concluded that fish eggs released on the shelf tend to stay there, with no tendency to drift into the inshore bays or onto the Grand Banks (see Fig. 1). When the effect of storms was included in their model, the transport of cod eggs into these bays was further reduced. They concluded that, under mean conditions of circulation, the northeast Newfoundland Shelf would be the nursery area of 2J3KL cod, not the inshore bays as previously described. Finally, Hutchings et al. (1993) evaluated historic records on cod spawning and concluded that spawning occurred not only along the outer banks but primarily over the NE Newfoundland Shelf as well as within the inshore bays. They suggested that inshore spawning may have contributed significantly to cod recruitment in coastal Newfoundland. Together, these observations indicate that spawning was much more broadly distributed than previously thought and that there is no simple mechanism of drift that ensures the inshore areas are the primary nursery areas.

Given the importance of this stock and the current depressed state of the fishery, there is interest in being able to predict cod recruitment both at an early age and independent of traditional data sources (Harris 1990). A better knowledge of the early life history is necessary before surveys can be designed to monitor juvenile cod abundance. There have never been synoptic surveys to describe the distribution and abundance of young cod in inshore and offshore areas. However, we did have available the results from several inshore surveys and one offshore survey during the fall of different years in which pelagic 0-group cod were taken. This pelagic occurrence is not unusual since 0-group cod in other areas occur in the water column for several months following spawning, settling to the bottom when around 7–8 cm in length (Perry and Neilson 1988). The purpose of this paper was to test the hypothesis that the majority of pelagic juveniles occur offshore rather than inshore as previously described. Our approach was twofold. First, we compared survey data from inshore and offshore during the month of September to provide a description of the distributions and abundances of pelagic 0-group cod in these areas. Second, using a simple model we estimated the timing of spawning in both areas and made first-order estimates of the relative abundances of cod eggs in the inshore and offshore areas.

Methods

Data sources

A total of four surveys were carried out in September of different years: offshore in 1981, inshore Trinity Bay in 1984 and 1985, and inshore Conception Bay 1989 (Fig. 1). The offshore survey was based on a systematic grid design where stations were approximately 54 km (30 nautical miles) apart. A total of 87 stations were sampled between 45°15′N and 50°15′N latitudes. This survey design is equivalent to a systematic sampling design (Snedcor and Cochran 1967), assuming that the first station was randomly selected from within a 54 × 54 km stratum. In Trinity Bay both the 1984 and 1985 surveys were based on a number of strata within which each station was selected randomly prior to the survey. In Conception Bay the sampling locations were based on a series of stations that had been selected to represent different sections of the bay.

All data were collected using an 8-m^2 rectangular midwater trawl (RMT-8) with a net mesh size of 4.5 mm and a cod end mesh size of 0.505 mm (Clark 1969). During the 1981 offshore survey, each tow was stepped obliquely

Table 1. Reported growth and mortality rates for cod larvae and juveniles used in the simulation model.

Area	Length (mm)	Growth rate ($mm \cdot d^{-1}$)	Data source	Reference
Irish Sea	5–22	0.49	Otoliths, Gulf III	Thompson and Harrop 1991
Browns Bank	3.6–43.0	0.44	Otoliths,	Campana and Hurley 1989
	3.6–24.8	0.30	bongos	
Flemish Cap	6.0–18.9	0.24	l-f,	Anderson 1982
	6.0–34.1	0.32	bongos	
	9.8–18.9	0.27		
Browns Bank	25	0.39–0.59	% growth rates, Tucker trawl and 1-m ring net	Suthers et al. 1989
Norway	45–90	1.61	l-f, Danish seine	Olsen and Soldal 1989

Area	Stage	Mortality rate (d^{-1})	Reference
Norway	Larvae	0.069–0.153	Sundby et al. 1989
	Juveniles	0.012–0.053	

Note: l-f refers to estimating differences in length frequencies for different periods of time.

from a 200-m depth to the surface with the net being towed for 5 min at depths of 150, 100, 50, 25, and 0 m at a speed of approximately 1.0–1.6 $m \cdot s^{-1}$ (2–3 knots) and at payout and retrieval rates of 0.5 $m \cdot s^{-1}$. Net depth was monitored with a net sonde transducer mounted on top of the net. When water depth was less than 200 m, then the tow was started from the next greatest depth, i.e., 150 m, 100 m, and so on. Total tow time was recorded for each tow. In the 1984 and 1985 inshore surveys a simple oblique tow was done to and from 200 m, or within 10 m of the bottom when bottom depth was less than 200 m. Otherwise, the sampling procedures were the same as in 1981. During the 1989 Conception Bay survey two simple oblique tows were done at each location to and from the 100-m depth, or within 10 m of the bottom, with one two being done towards the mouth of Conception Bay and the second tow towards the head. Abundances were averaged for the paired tows. All other sampling procedures remained the same in 1989 as in previous years.

All cod were removed from the samples, counted, and measured for total length to the nearest millimetre. Standardized estimates of abundance (N, fish·100 m^{-2}) were calculated as

$$[1] \quad N = \frac{C \cdot D}{8L} \cdot 100$$

where C is the number of cod collected, D is the maximum sampled depth (m), 8 is the area of the net opening (m^2), and L is the tow path (m). The tow path was estimated from tow time as

$$[2] \quad L = T \cdot S$$

where T is tow time (s) and S is ship speed ($m \cdot s^{-1}$).

In 1981 the sampling was carried out continuously over a 24-h period, but the inshore surveys were conducted only during daylight hours. To determine if there were day–night differences in catchability, day/night data for 1981 were analyzed for both standardized abundances ($cod \cdot m^{-2}$) and size (mm) of cod captured. In each case there were no statistical differences in either $\log_{10}$ abundances ($t = -0.8329$, $n_{day} = 43$, $n_{night} = 25$, $P = 0.4079$) or mean sizes ($t = -1.2284$, $n_{day} = 38$, $n_{night} = 24$, $P = 0.2241$).

Spawning back calculation model

A back calculation model was constructed in an attempt to make first order estimates of spawning times for pelagic juvenile cod captured in the fall, and to estimate the relative contributions of eggs spawned from inshore versus offshore locations. We know very little about the early life history of the Northern cod and begin with the question: did the juvenile cod sampled in September represent the primary survivors from the spring spawning or did we only sample larvae from the late spawning? If so, then back calculations would demonstrate a late spawning time and a low magnitude of abundance relative to the spawning stock. When comparing inshore to offshore abundances and relating these to back calculations of abundances to potential egg production based on stock assessment data we ask the question: did the abundance estimated for either the inshore or offshore areas represent the majority of eggs spawned in the spring? We recognize that many factors will affect growth and survival rates among areas and years. To evaluate possible variation we examined the output from three sets of parameter values. Variation in recruitment for the Northern cod varied by a factor of five during the 1980s (Baird et al. 1991). We are interested only in order of magnitude differences in output from the model that will allow us to evaluate the relative importance of the inshore and

Fig. 2. Distribution of pelagic 0-group cod sampled. (A) Offshore on the NE Newfoundland Shelf, Grand Bank, and St. Pierre Bank in September 1981 (number·100 m^{-2}). (B) Inshore in Trinity Bay in September 1984 (number·100 m^{-2}). (C) Inshore in Trinity Bay in September 1985 (number·100 m^{-2}). (D) Inshore in Conception Bay in September 1989 (number·100 m^{-2}). The Northwest Atlantic Fisheries Organization fisheries management zones for these areas are 2J, 3K, 3L, 3N, 3O, and 3Ps (shown in A).

Table 2. Maturity and fecundity for ages 3–14 used to estimate the numbers of eggs spawned each year by the Northern cod stock.

Age	Proportion mature	Fecundity
3	0	188 632
4	0.05	329 607
5	0.30	508 162
6	0.70	723 791
7	0.92	976 091
8	0.99	1 264 721
9	1.00	1 589 390
10	1.00	1 949 845
11	1.00	2 345 858
12	1.00	2 777 229
13	1.00	3 243 770
14	1.00	3 647 315

offshore areas based on the fall sampling data by relating this to known stock abundance data.

The model was dependent on stage-specific estimates of growth and mortality rates for larval and juvenile stages and temperature-dependent development and mortality rates for eggs. All rate estimates were taken from the literature (Table 1). Size at hatch was taken as 3.5 mm and metamorphosis from larvae to pelagic juvenile cod was set at 25 mm (Fahey 1983). Water temperatures used to estimate egg development and mortality rates were averaged for 0–20 m depth for eight different subareas that covered NAFO areas 3K and 3L for May and June, as summarized in Drinkwater and Trites (1986). Based on these back calculations, estimates of total egg abundance were calculated, one for each fall survey, by simple areal expansion of each area surveyed. Abundance for each station (number·m^{-2}) was assumed representative of that stratum (km^2) and total abundance for each stratum was expanded to the area of that stratum. Total abundance for each area was a simple sum of the strata abundance. Back calculation estimates were obtained by using different sets of parameter values for all growth, mortality, and development rates. An estimate of total eggs spawned in each survey year for the entire 2J3KL cod stock was based on the VPA estimate of numbers at age estimated each year (Baird et al. 1991, Table 52), maturity at age assuming a 50/50 sex ratio (C. Bishop, personal communication), and fecundity at age (May 1967) (Table 2).

The model used to back calculate the number of eggs spawned in the spring, based on the catch of juvenile fish in the fall was

$$[3] \quad N_0 = \sum_{i=1}^{n} N_i \prod_{j=1}^{3} e^{z_j t_j}$$

where N_0 is the number of eggs spawned, N_i represents the standardized number of juvenile fish in each length (mm) category i sampled, and j represents each life history stage such that 1 is juveniles, 2 is larvae and 3 is eggs. z represents instantaneous mortality rate for each stage duration t_j, where different juvenile and larval mortality values were selected from Table 1 and egg values were calculated as

$$[4] \quad z_e = 0.03 \exp^{0.18T}$$

where T is water temperature (Pepin 1991). t represents the time spent in each stage and is a function of growth rate g_i for juveniles ($j = 1$) and larvae ($j = 2$), and water temperature T for eggs, such that

$$[5] \quad t_1 = (J - 24)\cdot g_1^{-1}$$

$$[6] \quad t_2 = (L - 3.5)\cdot g_2^{-1}$$

$$[7] \quad t_3 = 74.131\cdot T^{-0.82}.$$

t_3 is after Page and Frank (1989). J is the size of juvenile cod captured at each station, where $J \geq 25$ mm and L is the size of larval cod for each back calculation, where 3.5 mm < L < 25 mm. Different growth rates for juveniles and larvae were selected from Table 1. The estimated spawning date distribution D_0 was calculated from each length category, as

$$[8] \quad D_0 = D_c - (t_1 + t_2 + t_3)$$

where D_c was the date of capture.

Results

Distribution

Pelagic cod were caught throughout much of the offshore area surveyed in September 1981 (Fig. 2A). These cod were concentrated in three areas: (*i*) a broad area overlying the NE Newfoundland shelf and the northern Grand Bank (NAFO areas 3KL); (*ii*) the southern Grand Bank (3NO); and (*iii*) near St. Pierre Bank (3Ps). There was some evidence of higher concentrations (≥10 cod·100 m^{-2}) for nearshore areas along the NE coast of Newfoundland as well as along the southern coast of Newfoundland. High numbers of cod were also observed at three stations offshore, demonstrating that high abundances were not confined to the nearshore areas.

Inshore, the range of abundances was similar to that observed offshore. In both 1984 and 1985 high abundances of cod (≥10 cod·100 m^{-2}) were observed near the head of the Trinity Bay (Fig. 2B and 2C). Throughout the bay the distribution of cod was irregular with catches ranging from 0 to >10 cod·100 m^{-2}. In 1985 the distribution of cod appeared to extend beyond the mouth of Trinity Bay, indicating that this distribution may have continued into the offshore area. In 1985 the sampling did not extend to the mouth, so inferences about offshore distribution cannot be made. Pelagic cod were caught at stations throughout

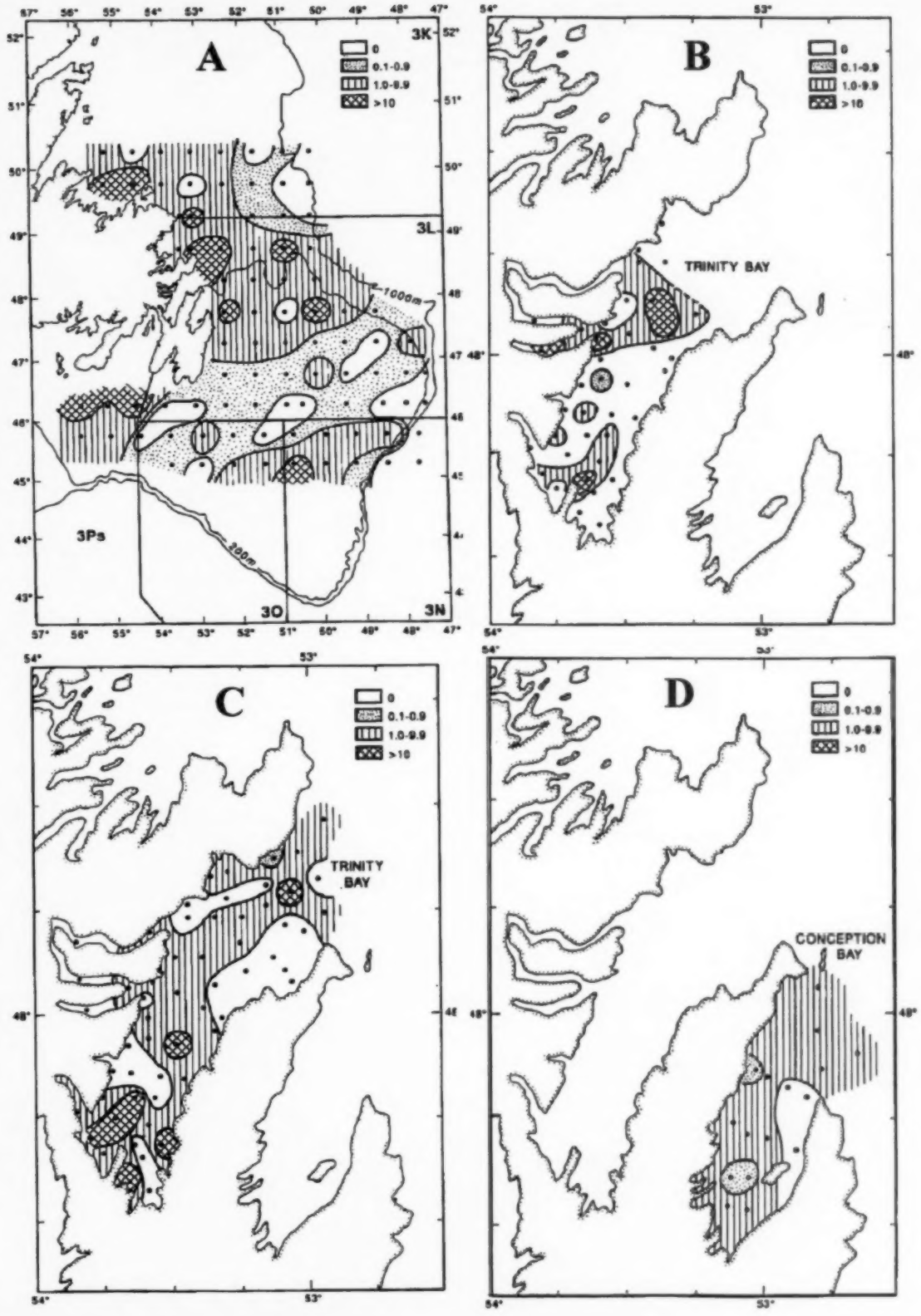

A
B
C
D
0
0.1-0.9
1.0-9.9
>10
3K
3L
3Ps
3O
3N
100m
1000m
TRINITY BAY
CONCEPTION BAY

Table 3. Summary of mean abundances and lengths of cod collected in offshore and inshore areas during September of different years.

Area	Year	Abundance ($100 \cdot m^{-2}$)	sd	*n*	Range ($100 \cdot m^{-2}$)	Length (mm TL)	sd	*n*	Range (mm TL)
Offshore	1981	6.4	10.16	62	0–56.6	37.6	14.11	467	17–70
Inshore TB	1984	8.2	9.47	16	0–27.1	36.5	12.35	79	7–67
Inshore TB	1985	11.0	23.19	32	0–130.7	28.4	12.79	114	4–65
Inshore CB	1989	4.3	3.27	17	0–12.9	34.1	15.67	41	12–69

Note: Offshore refers to the NE Newfoundland Shelf, Grand Bank, and St. Pierre Bank. TB refers to Trinity Bay and CB refers to Conception Bay.

Conception Bay in September 1989, although peak abundances were <10 cod·100 m^{-2} (Fig. 2D). Sampling indicated that abundances clearly extended beyond the mouth of Conception Bay. The only notable spatial pattern in abundances within the bay was the absence of fish on the eastern side.

Abundances

Pelagic 0-group cod caught offshore in 1981 averaged 6.4 cod·100 m^{-2} over all stations that captured cod while the maximum abundance was 56.6 cod·100 m^{-2} (Table 3). In Trinity Bay abundances averaged 8.2 cod·100 m^{-2} in 1984 and 11.0 cod·100 m^{-2} in 1985. Peak abundances were 27.1 and 130.7 cod·100 m^{-2}, respectively. In Conception Bay abundance averaged 4.3 cod·100 m^{-2} while the maximum abundance was 12.9 cod·100 m^{-2}.

Comparing abundances among inshore and offshore areas is constrained by sampling in different years for each area. Interannual differences in survival may affect the abundances of pelagic juveniles. In addition, juveniles sampled inshore may originate from local spawning that is not evaluated in the annual assessment of stock size. A comparison among the four cruises (offshore 1981, Trinity Bay 1984, 1985, and Conception Bay 1989) indicated there were no statistical differences, based on the nonparametric Kruskal–Wallis test ($\chi^2 = 5.01$, $P = 0.1714$, df = 3). Therefore, mean abundance sampled offshore in 1981 was not statistically different from the abundances in the two inshore areas sampled in three different years. The average inshore abundance (all three inshore surveys) was 7.8 cod·100 m^{-2}, which is similar to the mean of 6.4 cod·100 m^{-2} sampled offshore in 1981.

Sizes

In 1981 cod sampled offshore ranged from 17 to 70 mm total length and averaged 38.1 mm overall (Table 1). Where cod were abundant (i.e., >5 cod/tow) mean lengths ranged from 28.3 to 51.2 mm. The length frequency data indicated multiple peaks that ranged from 27 to 60 mm length, with apparent modes at 30, 45, and 52 mm length (Fig. 3). There was no apparent spatial pattern in mean cod lengths at different stations, nor was there a north–south (latitudinal) trend in mean length measured at each station (one way ANOVA $F = 1.2$, $P = 0.2776$, df = 61). In Trinity Bay mean lengths were 36.5 and 28.4 mm total length in 1984 and 1985, ranging from 7 to 67 mm and 4 to 65 mm, respectively (Fig. 3). In Conception Bay cod averaged 34.1 mm and ranged in length from 12 to 69 mm. Peaks in length occurred from about 25 mm up to 50–60 mm in length for each of the inshore surveys.

Levene's test for homogeneity of variances indicated a significant difference among the four data sets ($F = 4.78$, $P = 0.0027$, df = 637). A nonparametric test for differences in mean size among the four cruises indicated there was a statistical difference in mean sizes (Kruskal–Wallis test, $\chi^2 = 73.71$, $P = 0.0001$, df = 3). Wilcoxon's test for comparisons among pairs of cruises ($P < 0.05$) indicated that cod sampled in Trinity Bay in 1985 were smaller than those sampled in Conception Bay in 1989 and that both were smaller than cod sampled in Trinity Bay in 1984 and offshore in 1981, which were not statistically different from each other.

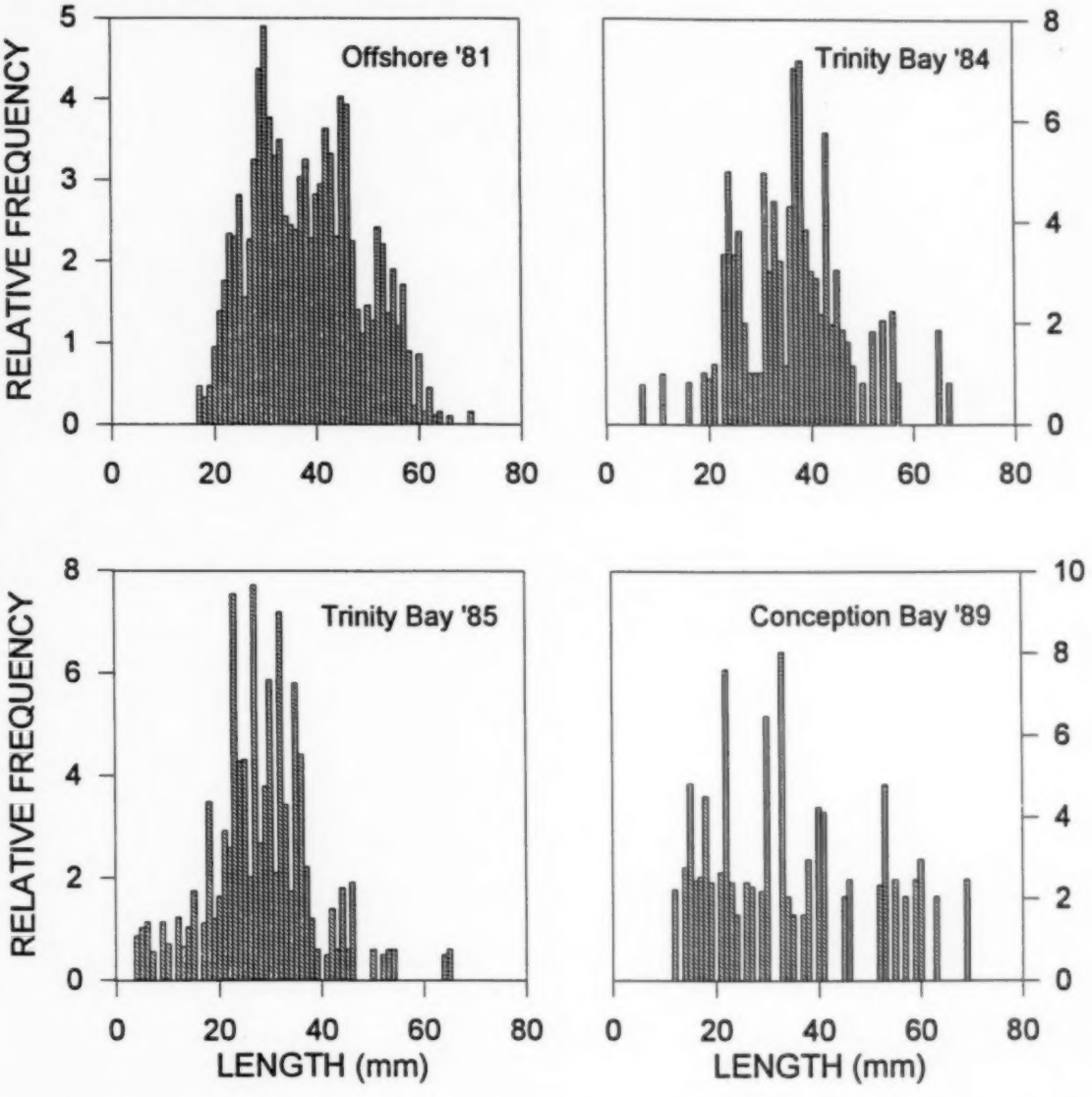
Estimates of spawning

Three sets of parameter values were used to estimate the time of spawning and the number of cod eggs spawned in the spring of 1981, based on the spawning back calculation model fit to the fall 1981 data. To represent average conditions, median values of temperature, growth, and mortality were used (Table 4). To bound the median estimate of spawning time, two sets of alternative parameter values were used. One represented slow growth and development of all three stages, which also were subjected to high mortality, defined here as "minimum." Alternatively a "maximum" parameter set was used representing fast growth and development with low mortality (Table 4).

Spawning was estimated to have occurred between 6 March and 2 June with a peak in spawning occurring on 23 April 1981, based on the median parameter values. Peak spawning occurred from early April (day 90) until late April (day 120) and was completed by the end of May (day 150) (Fig. 4). The minimum parameter set estimates that spawning occurred from 11 January to 12 February, while the maximum parameter set estimates that spawning would have occurred from 5 April to 17 June.

For comparison of spawning estimates between inshore and offshore areas only the median set of parameter values were used. From the data collected in Trinity Bay during September 1984, the model estimates that spawning occurred from the middle of March through June but was highest from mid-May extending into early June (Fig. 4). For Trinity Bay in 1985 spawning occurred from mid-May and extended through July. There were two periods of peak spawning, one occurring in late March and early April and the second during late May and June (Fig. 4). For Conception Bay in 1989 spawning was again estimated to

Fig. 3. Length frequency (%) distribution of cod sampled during September offshore in 1981 and inshore in Trinity Bay 1984, Trinity Bay 1985, and Conception Bay 1989.



occur from mid-March through July (Fig. 4). There were possibly two peaks of spawning, one in April and a second in late May – early June. However, numbers of cod from Conception Bay in 1989 were low and these estimates of peak spawning are based on a smaller number of fish compared with the other areas (Fig. 3).

From the 1981 data collected offshore for the area north of 47° latitude, the back calculations estimated that 6×10^{14} to 4×10^{19} eggs were spawned in 1981 for the minimum and maximum parameter sets, respectively. The median parameter set estimated that 5×10^{16} eggs were spawned. For the inshore areas, back calculations for the median parameter set estimated the total numbers of eggs produced were 2.1×10^{14} for Trinity Bay in 1984, 3.1×10^{14} for Trinity Bay in 1985, and 4.7×10^{13} for Conception Bay in 1989. Estimates of the number of eggs spawned each year based on maturity, fecundity, and VPA estimates of abundance at age were 1.3×10^{14} eggs in 1981, 1.3×10^{14} in 1984, 1.4×10^{14} in 1985, and 1.5×10^{14} in 1989.

Discussion

Inshore versus offshore

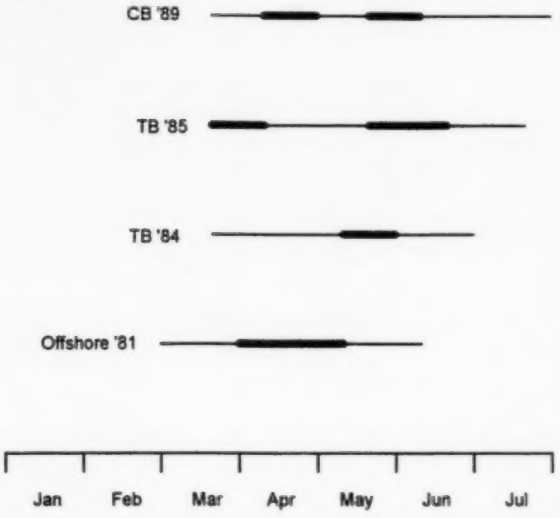
The existing early life history description for the Northern cod predicts that drift to inshore bays would have occurred within 40–60 days after spawning. As peak spawning is

Table 4. Parameter values used in simulations estimating the spawning period and population abundance of cod eggs spawned for the offshore area sampled in 1981.

	Eggs	Stage larvae	Juveniles
Median			
Temperature	2.81		
Growth		0.27	0.5
Mortality		0.111	0.033
Minimum			
Temperature	0		
Growth		0.24	0.40
Mortality		0.153	0.053
Maximum			
Temperature	6.0		
Growth		0.30	0.60
Mortality		0.069	0.012

Note: Temperature (°C), growth ($mm \cdot d^{-1}$), mortality (d^{-1}).

Fig. 4. Spawning periods (thin lines) estimated for each area and periods of peak spawning (thick lines) based on the back calculation model for the median parameter set. TB '84, Trinity Bay 1984; TB '85, Trinity Bay 1985; CB '89, Conception Bay 1989; Offshore '81, Offshore 1981.



expected to occur during April and May the majority of the cod eggs spawned offshore should have completed their drift by July–August at the latest. Evidence presented here indicates that cod eggs spawned offshore do not drift to the inshore areas along the NE coast of Newfoundland but remain offshore.

The abundance distribution of pelagic 0-group cod sampled in 1981 indicates that cod eggs spawned offshore that year largely remained offshore and did not drift to the inshore areas. This observation supports the model prediction of Helbig et al. (1992). High concentrations of pelagic juveniles were observed off Notre Dame Bay and Trinity Bay in 1981. Whether these distributions represented the results of drift from offshore spawning locations towards the inshore, or drift from bay spawning towards the offshore, cannot be determined, as sampling did not extend between inshore and offshore areas. Sampling inshore in other years demonstrated that concentrations of cod were not different from those observed offshore in 1981. In Trinity Bay highest concentrations were observed near the head of the bay each year. The occurrence of these high concentrations is consistent with high numbers of juvenile cod sampled near beaches over a large geographic area in previous years (Lear et al. 1980) and in Trinity Bay in recent years (D. Methven, Memorial University of Newfoundland, personal communication).

While mean abundances, measured as number per square metre, were not different between the inshore and offshore areas, the offshore area was much larger resulting in higher absolute abundance. Trinity and Conception Bays represent approximately one-fifth of the inshore area along the NE coast of Newfoundland. Therefore, as an average of the three years sampled, we estimate that only 1.5×10^{15} eggs would be represented by all inshore areas compared with 5×10^{16} for the offshore area sampled in 1981 (north of 47°N). If we assume the extreme case that Trinity and Conception Bays only represent one-tenth of all inshore areas, our estimate of total egg abundance for the all inshore areas is still <10% of that estimated from the offshore area in 1981. In addition, the offshore area surveyed in 1981 did not extend north of 50°15′N latitude, although the distributions indicated high abundances occurred north of the survey area. Therefore, our estimate of total eggs spawned offshore must be conservative.

There is a notable difference between the estimates of total egg abundance based on back calculations from the pelagic 0-group survey in the fall of 1981 and those based on VPA estimates of abundance. In the back calculation model, the median parameter set corresponded closely with the expected duration and time of peak spawning for this population, but estimated egg abundance was more than two orders of magnitude greater. The fact that the simulation tended to overestimate the number of eggs spawned in the spring could result from several factors: (*i*) egg, larval and (or) juvenile mortalities could have been overestimated in the model; (*ii*) the standardized estimates of juvenile cod abundance ($cod \cdot 100\ m^{-2}$) are too high; (*iii*) maturity and (or) fecundity estimates for adult cod are too low. When comparing inshore with offshore areas we used the median parameter set to estimate egg abundances spawned. Differences in survival between areas would have to be on an order of magnitude, or greater, to discount the offshore data from 1981. Currently, there is no information to support such differences (see below).

Finally, comparison of cod sizes caught each year in the inshore and offshore areas does not support a large scale drift of cod from offshore to inshore areas. Assuming similar growth rates, these cod would be approximately the same age. If there was a large scale downstream transport of cod eggs and larvae to inshore areas, then cod sampled

inshore should have been significantly larger and older compared with those offshore. In fact the opposite was observed: cod were significantly smaller inshore in 2 of 3 years compared with those offshore in 1981.

We suggest that, at most, only part of the population of cod eggs spawned offshore would have drifted to inshore areas. Alternatively, pelagic cod observed inshore most likely originated from eggs spawned inshore. It has been reported that coastal populations of cod exist and that their contribution to recruitment may be considerable (Hutchings et al. 1993). Templeman (1979, 1981) reported that inshore spawning was generally later than that offshore. This is supported by the back calculations of spawning times that were later for the inshore areas, both as total duration and the consistent late peaks in spawning compared with offshore (Fig. 4).

Stock structure

In 1981 pelagic 0-group cod were observed abundantly offshore in three different areas. These distributions are consistent with the existing management units for different cod stocks: the Northern cod stock complex of southern Labrador, the NE Newfoundland Shelf and Northern Grand Bank (2J3KL); the southern Grand Bank cod stock (3NO); and St. Pierre Bank cod stock (3Ps). The distributions imply that offshore spawning in 2J3KL results in a broad distribution over the southern NE Newfoundland Shelf and northern Grand Bank at the pelagic juvenile stage (5–6 months of age). Unfortunately, sampling did not include the northern area of 3K nor any of area 2J. High concentrations of cod (1–10 cod·100 m^{-2}) observed offshore over much of the NE shelf appear to have extended north of the surveyed area. However, future studies will be necessary to determine the northern extent of this distribution. On the southern Grand Bank it is reasonable to expect that cod eggs spawned there will be retained there as pelagic juveniles and eventually settle to the bottom. At 1 year of age, cod are found abundantly over the southern Grand Bank (S. Walsh, Northwest Atlantic Fisheries Center, St. John's, Nfld., personal communication). Studies on capelin and flatfish have demonstrated that eggs spawned on the southern Grand Bank are retained there as larvae (Frank and Carscadden 1989; Frank et al. 1992). Finally, while little is known about spawning and drift on St. Pierre Bank, the discrete distribution of pelagic juveniles is consistent with the retention of eggs and larvae over this bank.

North–south spawning cline

Offshore, the lack of a significant north–south cline in cod sizes is not consistent with reports that cod spawn earliest in the north and later in the south (Myers et al. 1993). Two facts could account for the similarity of mean sizes observed among all stations sampled offshore in the fall of 1981. One is that cod spawned in the north grew slower so that mean sizes of all cod in the sampling area were the same in the fall, following several months of growth. Alternatively, a similar size distribution would occur if there was a downstream transport of cod from north to south over the NE shelf. Helbig et al. (1992) predicted that cod eggs spawned offshore would be transported south, but not off the NE shelf. This would result in an accumulation and mixing of pelagic cod over the southern area of the NE shelf and the northern Grand Bank, thus obscuring any differences in ages or growth rates.

Spatial and temporal variability

Interannual differences in the distributions of 0-group juvenile cod will occur due to natural factors. Suthers and Frank (1989) reported different distributions of pelagic juvenile cod in different years sampled, and they cautioned about generalizing from 1 year's sampling. For a single year Polacheck et al. (1992) reported the large scale transport of haddock eggs and larvae significantly away from the traditional area of distribution as juveniles. Interannual differences could result from variations in spawning locations, ocean transport, stock size, and differential survival of eggs–larvae–juveniles among locations. Primary factors resulting in differential survival would include differences in temperature, food availability, and ocean transport. All of these factors can be expected to operate during a given year, effecting an outcome on the distribution and abundance of pelagic 0-group cod. While acknowledging that annual differences will occur, we are concerned here with testing the hypothesis that the majority of the cod eggs spawned in the offshore area of 2J3KL remain offshore and are not transported to the inshore areas. Ultimately, the interaction of the prime factors controlling distributions cannot be assessed for the years in which we have data. We can ask, however, if there are obvious indications that any single factor deviated markedly during these years.

Spawning location offshore may have undergone a dramatic southward shift in recent years and is predicted to have a significant effect on the distribution and survival of the eggs spawned (deYoung and Rose 1994). However, this spawning shift apparently did not occur until the collapse of the stock in the 1990s and would not have affected the offshore distribution observed during most of the 1980s, particularly in 1981.

Ocean transport may have varied during these years but we have no direct measurement of this. The region is ice covered during much of the period in which eggs and larvae are passively drifting. However, Helbig et al. (1992) modelled the effect of storms on the mean dispersal of cod eggs spawned offshore and found that, if anything, there was a reduced transport inshore. Only under conditions of "favourable" onshore forcing would significant numbers of cod eggs and larvae be transported inshore. At present, there is no indication that his has occurred.

Stock size fluctuated by a factor of two during the period 1980–1990, being highest in 1985 (Baird et al. 1991). Stock abundance in the other years compared with 1985 was 0.67 in 1981, 0.94 in 1984, and 0.89 in 1989. Assuming there were no significant changes among years in age of maturity and fecundity, then the estimated number of eggs spawned was smallest in 1981. If the number of eggs spawned each year is reflected in abundance at the pelagic juvenile stage, then we would consider the offshore distribution in 1981 to represent a "minimum" occurrence.

Alternatively, significant variations in natural mortality may decouple a simple relationship between the number of eggs spawned in the spring and pelagic cod abundance in the fall. Recruitment (age 4) varied by a factor of 3.3 during

the period 1980–1990, being highest for the 1986 year class (Baird et al. 1991). For the years considered in our study, the 1981 year class was the largest (0.95 compared with 1986), followed by 1984 (0.36) and 1985 (0.61). Recruitment data are not available yet for 1989. Given that mean abundances between offshore (1981) and inshore (1984, 1985) were not significantly different at the pelagic juvenile stage, and the 1981 year class was stronger at age 4, it is clear that offshore distribution in 1981 was not detrimental to survival.

Differential survival may occur between inshore and offshore locations during any given year, or across all years and most likely would occur due to differences in temperature, food availability, and (or) predatation. This is impossible to investigate for our area based on existing data. We do know that there is a large scale coherence of temperatures throughout the offshore area (Petrie et al. 1992), which would tend to minimize differences in growth and survival due to temperature. While temperatures differ, on average, between the inner and outer shelves, the magnitude is typically <1°C (Drinkwater and Trites 1986). Similarly, differences in temperature between Conception Bay and the offshore is typically <2°C (B. deYoung, Physics Department, Memorial University of Newfoundland, St. John's, Nfld). If warmer temperatures are critical in driving differences in growth rates (as may affect our estimates of spawning times) or survival (which may affect our estimates of abundances between areas), then the inshore area may be more favourable, given the higher average temperatures. While temperature has been assumed to have a direct effect on growth rates in larval fish (Campana and Hurley 1989), Anderson (1994) has shown that temperature did not have a direct effect on growth rates of larval redfish, growth being driven instead by food availability. In addition, there can be a trade-off between growth and mortality rates where both rates increase at higher temperatures (Houde 1989; Pepin 1991). Such an effect would minimize differences between areas due to temperature.

Our back calculation model estimates differences in abundance between inshore and offshore areas to be an order of magnitude, and greater. At present, there is no indication that factors responsible for differences on this order occurred between inshore and offshore areas during the years of this study. The extensive distribution of pelagic cod sampled offshore in 1981 is compelling evidence that the offshore is an important habitat at this point in their life cycle. While there may be variable survival between areas among years we conclude that historically the offshore area has been the primary habitat for pelagic 0-group cod.

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A wall jet to measure the attachment strength of zebra mussels

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Abstract: A wall jet is presented as a novel means of measuring the attachment strength of zebra mussels. Attachment strength was inferred from a fluid detachment parameter (DP), defined as the nominal wall shear stress at the detachment site × mussel length^2. DP varied significantly on natural and artificial substrates: in tests with 288 *Dreissena bugensis* (≈8–10 mm long), the mean (±SE) DP was 8.9 ± 0.9 mPa·m^2 on limestone/dolomite, 5.6 ± 0.5 mPa·m^2 on polyvinylchloride, 4.3 ± 0.4 mPa·m^2 on stainless steel, 4.2 ± 0.5 mPa·m^2 on aluminum, and 2.5 ± 0.3 mPa·m^2 on polymethylmethacrylate (Plexiglas). The attachment strength of postlarval mussels (plantigrades; <1 mm) was two orders of magnitude less than adult mussels. These results were validated with conventional tensile loadings, in which 633 *Dreissena bugensis* and 26 *Dreissena polymorpha* were pulled off substrates with a calibrated force scale. The tensile loadings results were comparable with those of marine bivalves. Good correlation between pull-off force and DP was observed. Information of this nature is useful for the implementation of environmentally benign zebra mussel controls.

Résumé : Nous présentons un gicleur qui constitue un nouvel outil pour mesurer la force d'attachement des moules zébrées. La force d'attachement est déduite d'un paramètre de détachement hydraulique (DP), défini comme la contrainte nominale de cisaillement au point de détachement × longueur de la moule^2. Le paramètre DP variait de façon notable selon les différents substrats, naturels et artificiels : dans des essais menés sur 288 *Dreissena bugensis* (≈8–10 mm de longueur), le DP moyen (±É-T) était de 8,9 ± 0,9 mPa·m^2) sur calcaire/dolomite, 5,6 ± 0,5 mPa·m^2 sur chlorure de polyvinyle, 4,3 ± 0,4 mPa·m^2 sur acier inoxydable, 4,2 ± 0,5 mPa·m^2 sur aluminium et 2,5 ± 0,3 mPa·m^2 sur polyméthacrylate de méthyle (Plexiglas). La force d'attachement des postlarves de moules (plantigrades; < 1 mm) était de deux ordres de grandeur inférieure à celle des moules adultes. Les résultats ont été validés par des essais d'effort de traction classiques, dans lesquels 633 *Dreissena bugensis* et 26 *Dreissena polymorpha* ont été arrachées de leurs substrats selon une échelle de force étalonnée. Les résultats des essais de traction étaient comparables à ceux obtenus sur des bivalves marins. On a observé une bonne corrélation entre la force d'arrachement et le paramètre DP. L'information de cette nature est utile pour la mise en oeuvre contre la moule zébrée de moyens de lutte qui ne nuisent pas à l'environnement.
[Traduit par la Rédaction]

Introduction

The adhesion of epibenthic organisms is relevant to many oceanographic and (to a lesser extent) limnological disciplines including biology, ecology, and engineering (Waite 1983; Mihm and Loeb 1992). Nowhere is this more evident than in the case of zebra mussels (*Dreissena polymorpha* and *Dreissena bugensis* (May and Marsden 1992; Domm et al. 1993; Rosenberg and Ludyanskiy 1994)). The ecological and economic impacts caused by zebra mussel "fouling" are well known (Hebert et al. 1989, 1991; Roberts 1990; Griffiths et al. 1991; O'Neill and MacNeill 1991; Hunter and Bailey 1992; Nalepa and Schloesser 1993; Claudi and Mackie 1994). Unfortunately, these impacts will continue with the rapid geographic expansion of the mussels (Neary and Leach 1992; Anonymous 1994).

A better understanding of the mechanisms and mechanics of zebra mussel adhesion is being gained through research (Ackerman et al. 1992; Baier et al. 1992; Rockhill and Bonner 1992; Eckroat et al. 1993) and comparisons with other, mostly marine, bivalves (e.g., Waite 1983). This information may also provide insight into environmentally

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benign zebra mussel control and mitigation strategies. However, there remains a dearth of information on the attachment strength of zebra mussels on natural materials, and only a few man-made materials have been examined (e.g., Ackerman et al. 1992). Such information would allow "easily cleaned" materials to be incorporated into structures expected to be exposed to zebra mussels.

Furthermore, while there are a number of attachment strength measurement techniques (review in Ackerman et al. 1993), few can be used with small organisms (e.g., postlarval zebra mussels) or organisms that cannot be easily gripped by conventional testing devices (e.g., algae, sponges, bryozoans, etc.). Moreover, in many situations it is useful to monitor the detachment process under "natural", nonintrusive conditions. To meet these needs, we designed and constructed a wall jet device to measure attachment strength in a relatively nonintrusive and convenient manner. In this report, we describe the wall jet apparatus and its use to characterize the attachment strength of zebra mussels on natural and man-made materials.

Materials and methods

Wall jet apparatus

A wall jet apparatus was designed and constructed to generate fluid forces sufficient to detach zebra mussels from substrates (Fig. 1). The wall jet is produced when a jet of fluid leaves a duct and impinges tangentially on a stationary wall within a quiescent body of fluid (Glauert 1956; Bakke 1957; Sigalla 1958; Schlichting 1979; Fujisawa and Shirai 1989). Measurements can be made close to the duct outlet, where the fluid dynamic properties (e.g., velocity distribution, wall shear stress, etc.) of the wall jet are determined by the boundary layer forming on the wall. This provides a reasonably well-defined fluid environment in which to study the adhesion or attachment strength of organisms. Further, the flow characteristics within the wall jet can be conveniently altered by changing the jet flow rate.

In our system, lake water was pumped from a polyvinylchloride (PVC) reservoir, through 2-in. (5.08-cm)-diameter PVC water pipe across a 3.048-cm-diameter orifice plate flow meter and into a 1-m-long rectangular duct (2 × 3 cm internal cross section; Fig. 1). Flow was regulated with a 2-in. (5.08-cm) globe valve downstream of the orifice plate flow meter and was determined by differential pressure measurements across the previously calibrated orifice plate flow meter. The duct emptied into a water-filled holding chamber at a 3° angle to the horizontal, creating a wall jet that impinged on a coupon (8 × 16 cm) placed at the outlet of the duct. To facilitate access to mussels in different locations on the coupon, the duct was positioned in one of two configurations: (1) in the *down* position, the upper surface of the bottom wall of the duct was flush with the coupon, and (2) in the *up* position, the duct rested on the surface of the coupon with the upper surface of the bottom wall 1 mm above the coupon (Fig. 1B). Water returned to the reservoir by draining out of the holding tank, over a gate. The holding tank was fitted with glass viewing ports to permit video microscopy. The positioning of mussels and coupons are described below.

To characterize the fluid dynamics of the wall jet at each flow rate, the wall shear stress (τ_w, pascals) was determined in both the duct-up and duct-down configurations using a modified Preston tube (Winter 1977; Nitsche et al. 1985), described in detail in Ackerman et al. (1994). Seventy-five measurements of τ_w were made at a grid of (x,y) locations on the surface of a single smooth coupon, where x is the downstream position from the duct outlet and y is the transverse position measured from the duct centerline. Measurements in the interval $0 \le x \le 13$ cm (14 cm in the duct-down configuration), $0 \le y \le 3$ cm were used to generate contours of τ_w for each flow rate used in this study. As well, the shear stress on the centerline of the lower wall of the duct 1.4 cm upstream of the duct outlet (i.e., within the duct) was determined using the Preston tube.

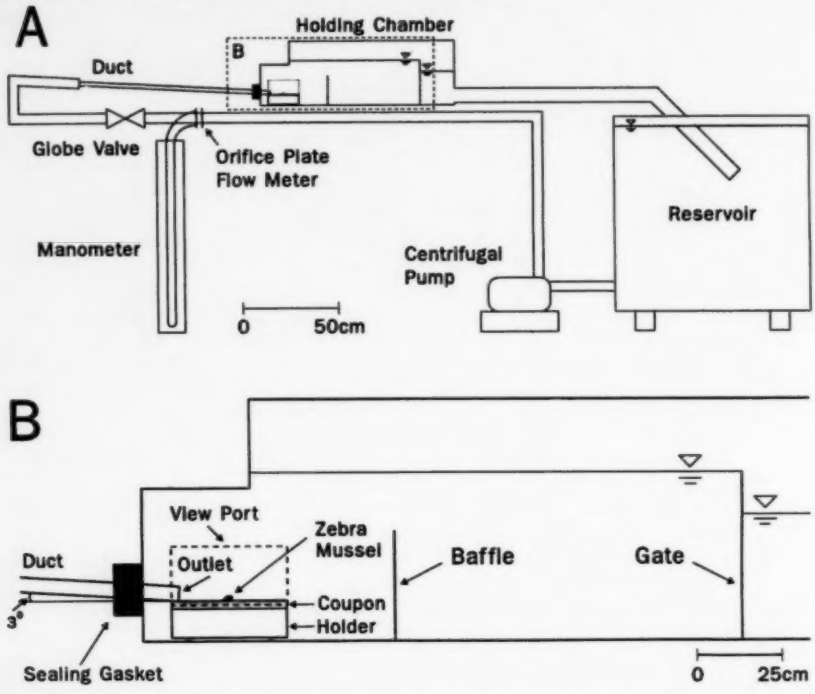
In the present work, shear stress contours measured on a smooth coupon were taken to be representative of the shear stresses which would exist on all coupons, independent of coupon roughness. The smooth coupon shear stress contours were therefore used to determine the nominal shear stress acting on zebra mussels during the detachment experiments (see below).

Experimental protocol

Coupons (8 × 16 cm) of PVC, polymethylmethacrylate (PMMA; =Plexiglas), 304 stainless steel (SS), and 6066-T6 aluminum (AL) were deployed at a minimum depth of 1.5 m in the forebay of Ontario Hydro's Nanticoke Thermal Generating Station, Nanticoke, Ont., (NTGS; Lake Erie) in May 1991. A number of coupons with postlarval mussels (plantigrades; <1 mm) were tested in September 1991. Unfortunately, the rapid growth of the mussels precluded our testing of additional postlarvae, and we therefore tested small adults (>7 mm long). These were collected in the winter, spring, and summer of 1992. Fortunately, mussels of this size could be gripped in a tensile loading device to permit independent validation of the wall jet measurements. At the time of collection, lake water and coupons or rock samples (limestone 85% and dolomite 15%; Niagara Escarpment; Hewitt and Vos 1972) from a minimum forebay depth of 1 m were transported from NTGS to the laboratory where they were maintained at 8–12°C for up to 1 month. During that time the substrates (rocks and coupons) were kept in aerated lake water aquaria under a 12 h light: 12 h dark cycle with constant filtration. Mussels attached to substrates were fed a mixture of live *Chlorella emersonii* and *Selenastrum capricornutum* (UTCC-86 and UTCC-37, respectively; University of Toronto Culture Collection, Department of Botany). Water changes were conducted at least once a week and the dissolved O_2 and pH of each aquarium were monitored closely.

Except where stated, the zebra mussels examined in this study, and the majority of mussels (i.e., >90%) that recruited in our field site, were *D. bugensis* (the quagga mussel), which has a tapered ventral surface (May and Marsden 1992; Domm et al. 1993; Rosenberg and Ludyanskiy 1994). The "typical" zebra mussel (*D. polymorpha*) has a flat, slightly concave ventral surface. Voucher specimens of "typical" (ROMIZ M8682) and "quagga" (ROMIZ M8683) species of zebra mussels have been deposited in the Department of Invertebrate Zoology of the Royal Ontario Museum (Toronto).

Fig. 1. (A) Scale drawing of the wall jet apparatus used to measure adhesion strength of zebra mussels and (B) details of the duct and chamber.



Prior to an experiment, substrates were removed from the maintenance facility and allowed to acclimatize to room temperature (15–17°C) overnight, or for a minimum of 3 h. As a result of the strong recruitment of larvae in 1991, the majority of mussels, which formed a multiple layer on the substrates, were carefully removed from the substrates to provide relatively isolated mussels for wall jet testing. This was desirable to avoid mussel–mussel fluid–mechanical interactions. Mussel removal (i.e., detachment from the substrate) was accomplished by holding the mussel in forceps or in the spring-loaded grips of a precision scale (100-, 300-, and 500-g capacity; Pesola, Switzerland) and pulling normal to the face of the substrate. In the case of mussel removal using the force scale, the detachment force (DF, newtons) was recorded so as to allow a comparison of direct mussel pull-off data with the wall jet measurements and to compare zebra mussel adhesion with published results from other species. The load was applied at a constant rate (e.g., $\approx 0.5\ N \cdot s^{-1}$ for 100-g scale). Approximately 10–15 isolated mussels remained on a substrate coupon after the mussel removal protocol.

Most wall jet tests were carried out with the duct in the "down" configuration (interior surface of duct lower wall flush with coupon surface). Care was taken to ensure that the portion of the substrate to which a mussel was attached was level so as to maintain the 3° angle between substrate and duct. The substrate was shifted until the mussel under investigation was within $y = 1.0$ cm of the centerline of the duct, and its x and y positions were noted. The initial orientation of each mussel, with respect to the opening of the duct, was also recorded.

Water flow through the wall jet was increased incrementally until mussel detachment was observed and recorded using video microscopy. More specifically, beginning at time zero, the flow control valve was opened slowly (e.g., $\approx 1.38 \times 10^{-4}\ m^3 \cdot s^{-2}$) to a flow rate of $1.38 \times 10^{-3}\ m^3 \cdot s^{-1}$ corresponding to a 3-cmHg (1 cmHg = 1.33 kPa) differential pressure across the orifice plate flow meter. This flow rate was maintained for 30 s, after which the flow was again increased by a differential orifice plate pressure of 3 cmHg. This procedure was repeated until the mussel detached, or until the maximum flow rate of $4.5 \times 10^{-3}\ m^3 \cdot s^{-1}$ was reached. The adhesion strength of zebra mussels was inferred from the fluid detachment parameter (DP, millipascals × square metre) calculated from the product of the nominal wall shear stress (τ_w) at point of detachment and the square of the mussel length (l, metres; i.e., $DP = \tau_w \cdot l^2$).

Results

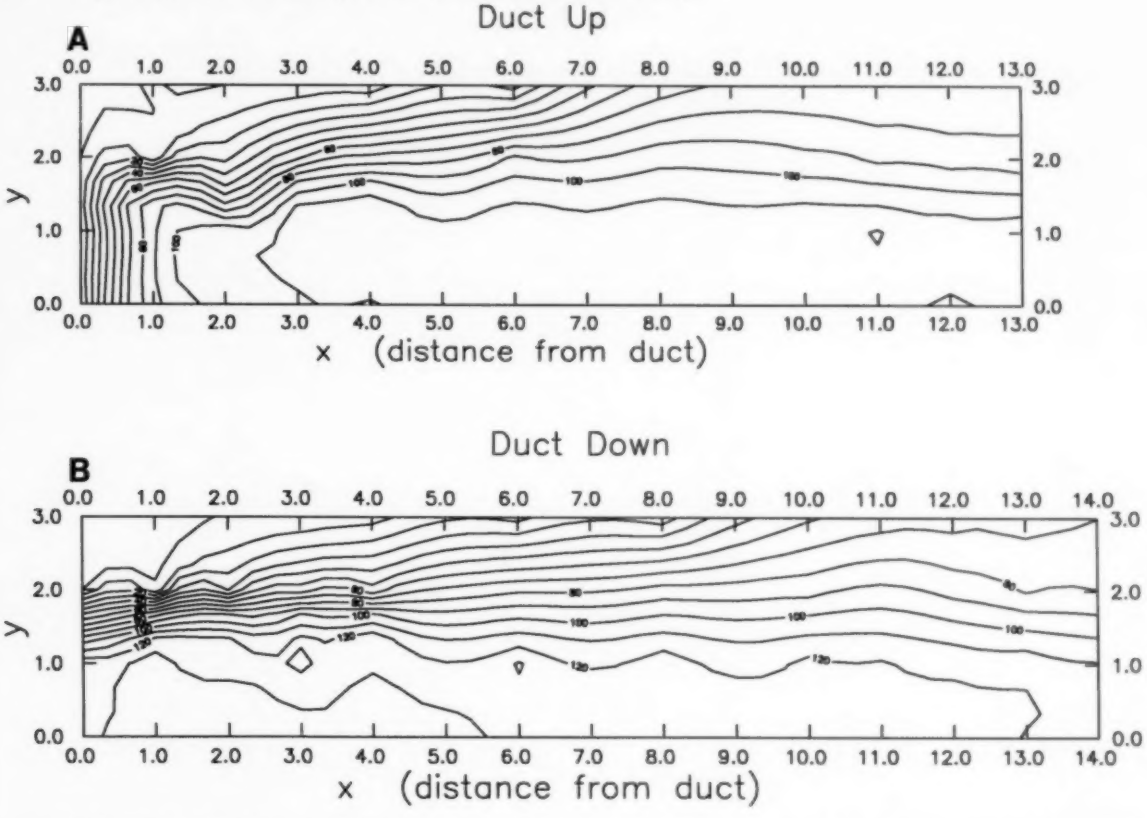
Fluid dynamic characteristics of the wall jet

Based on calibration tests for the orifice plate flow meter, the average velocity in the duct ranged from $2.3\ m \cdot s^{-1}$ at an orifice plate differential pressure of 3 cmHg to $7.5\ m \cdot s^{-1}$ at 33 cmHg. This corresponds to duct-corrected Reynolds numbers, Re_{duct}, from 6×10^4 to 2×10^5 where

$$[1] \qquad Re_{duct} = \Phi \cdot \frac{d_h \cdot V}{\nu}$$

and $\Phi = 1.074$ is the form coefficient of the duct (Jones 1976), d_h is the duct hydraulic diameter (metres), V is the average fluid velocity in the duct (metres per second), and

Fig. 2. Wall shear stress (τ_w, Pa) contours on the surface of a smooth coupon at a flow rate of 4.5×10^{-3} $m^3 \cdot s^{-1}$ for (A) duct-up and (B) duct-down configurations (x and y are expressed in cm).



ν is the kinematic viscosity (square metres per second). Based on these values of Re_{duct}, the flow was fully developed and turbulent at the outlet of the duct. The measured centerline wall shear stress (τ_w) within the duct ($x = -1.4$ cm, $y = 0$ cm) ranged between 13.3 and 117 Pa for this range of flows.

The τ_w contour maps at different flow rates shared a number of common features (Fig. 2). The highest τ_w values were found at the center of a coupon several centimetres downstream of the duct outlet. A steep cross-stream gradient in τ_w was observed at $y \approx 1.5$ cm close to the duct ($x = 0$ cm) and at greater y distances as the distance from the duct increased. This is what would be expected as the 3-cm-wide jet gradually grows in width as x increases. Differences in τ_w maps between the two duct positions were noted, including (1) generally higher τ_w values in the down configuration and (2) a steep downstream gradient in τ_w between $x = 0$ cm and $x \approx 1.5$ cm in the duct-up configuration.

Zebra mussel adhesion

The detachment of mussels from substrates using the wall jet depended on the original orientation of a mussel relative to the duct. As soon as flow was initiated, mussels lifted slightly off the substrate, indicating that they were being held in place by tension in their byssal threads. Generally, when flow impinged anywhere except directly on the left or right valves, mussels twisted about their byssus until they were perpendicular to the flow (i.e., so that the flow was directed towards the valves). As the flow was increased, they began to oscillate (≈7 Hz) about their byssal connections in the plane of the surface and finally detached from the surface. While not measured in this study, detachment occurred through a combination of three mechanisms: (1) failure of the byssal pad, (2) failure of the byssal thread, and (3) failure of the byssal stem at the insertion of the retractor muscle. The detachment event was rapid and generally occurred within 30 s of an increase in the jet flow rate. Once detached, a mussel moved downstream and upwards from its original position. Some mussels formed new byssal attachments to the walls of the reservoir tank.

Based on tests with 288 mussels, the DP required to remove zebra mussels from substrates ranged from 0.01 to 29.4 mPa·m^2 (Table 1A). In some cases (16% for rocks; 2–4% for coupons), the fluid forces were not sufficient to remove mussels within the 30-s exposure time at a maximum flow rate of 4.5×10^{-3} $m^3 \cdot s^{-1}$. These mussels were not included in the subsequent statistical analysis. Mussel attachment strength, as inferred from DP, was greater on rocks (8.9 ± 0.9 mPa·m^2; mean ± SE) than on the man-made coupons. On coupons, the rank of attachment strength was PVC (5.6 ± 0.5 mPa·m^2), SS (4.3 ± 0.4 mPa·m^2), AL (4.2 ± 0.5 mPa·m^2), and PMMA (2.5 ± 0.3 mPa·m^2).

Table 1. Attachment strength of zebra mussels (*D. bugensis* and *D. polymorpha*) on different materials under different measurement techniques. All values are reported as mean ± SE.

A: Results from the wall jet

Substrate type	Detached mussels (*n*)	Mussel length (mm)	Fluid detachment parameter (mPa·m^2)		Undetached mussels	
			Mean ± SE	Range	*n*	% total
Mussels (*D. bugensis*)						
Rocks	55	10.4±0.4	8.9±0.9	0.5–29.4	11	16
PVC	58	8.7±0.3	5.6±0.5	0.01–14.0	2	3
PMMA	53	7.7±0.2	2.5±0.3	0.2–9.8	1	2
AL	28	8.7±0.3	4.2±0.5	0.3–9.3	1	3
SS	73	7.9±0.2	4.3±0.4	0.5–14.8	3	4
Postlarval mussels (i.e., plantigrades)						
PVC	13	0.5±0.1	0.02±0.01	0.02–0.17	0	0
SS	8	0.8±0.1	0.05±0.02	0.05–0.10	0	0

B: Results from tensile loading tests

Substrate type	Detached mussels (*n*)	Mussel length (mm)	Detachment force (N)		Undetached mussels	
			Mean ± SE	Range	*n*	% total
Mussels (*D. bugensis*)						
Rocks	324	10.4±0.2	1.55±0.07	0.12–9.80	0	0
PVC	159	9.3±0.1	0.87±0.04	0.11–2.94	0	0
PMMA	38	8.8±0.2	0.35±0.03	0.12–0.98	0	0
AL	36	8.1±0.2	0.47±0.05	0.12–1.27	0	0
SS	76	8.1±0.2	0.67±0.06	0.03–2.58	0	0
Mussels (*D. polymorpha*)						
Rocks	26	10.0±0.8	1.4±0.4	0.2–8.8	0	0

The DP values for postlarval mussels (presumably >90% *D. bugensis*) were considerably lower, averaging 0.02 ± 0.01 mPa·m^2 on PVC and 0.05 ± 0.02 mPa·m^2 on SS (Table 1A). Not surprisingly, there was a significant regression of DP on mussel length for all substrates.

As measured by the direct pull-off experiments of 633 mussels, the tensile load required to detach mussels from substrates (DF) ranged from 0.03 to 9.8 N. There were no cases in which mussel attachment strength was greater than the scale maximum of 9.8 N. The attachment strength inferred from the DF was positively related to mussel length among rocks ($r^2 = 0.57$; $P < 0.001$), PVC ($r^2 = 0.43$; $P < 0.001$), SS ($r^2 = 0.30$; $P < 0.001$), and AL ($r^2 = 0.19$; $P < 0.01$). The average attachment strength was greatest on rocks (1.55 ± 0.07 N), followed by PVC (0.87 ± 0.04 N), SS (0.67 ± 0.06 N), AL (0.47 ± 0.05 N), and PMMA (0.35 ± 0.03 N). The attachment strength of *D. polymorpha* on rocks was 1.4 ± 0.4 N (Table 1B), which was comparable with the strength of *D. bugensis*. Unlike the results from the wall jet experiments, these results were not normally distributed ($P < 0.05$).

Discussion

Wall jet characterization

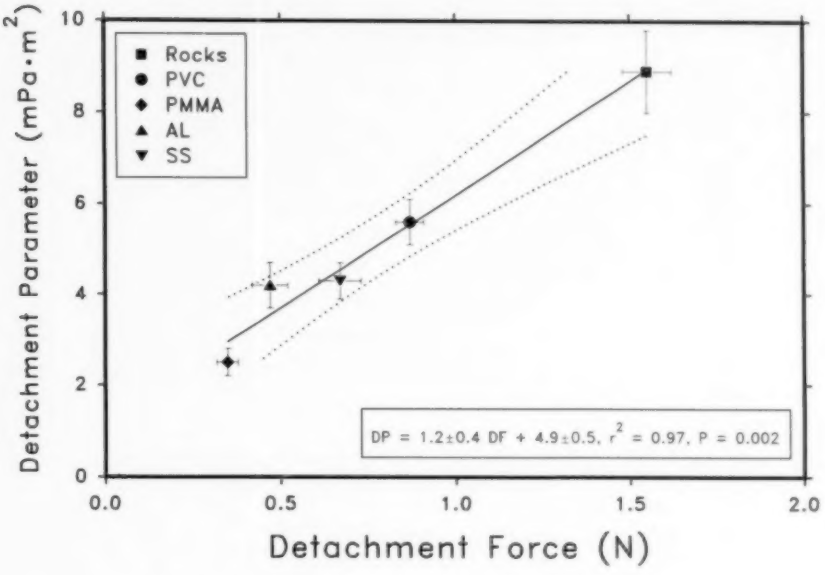
The measured fluid dynamic characteristics of flow within the duct agreed well with results of other empirical studies of flow in rectangular ducts of differing aspect ratio (Dean 1978; Knight and Patel 1983; Leutheusser 1984). In our case, the expected centerline wall shear stress on the lower surface of the duct (aspect ratio of 1.5) is given by

$$[2] \qquad \tau_{CL} = 1.09 \cdot \frac{f_\Phi}{8} \cdot \rho \cdot V^2$$

where f_Φ is the friction factor corrected for duct flow (see Eq. 1) and ρ is the fluid density (kilograms per cubic metre) (Knight and Patel 1983). The τ_w measured within the duct was on average within 1.1% of the values predicted by Eq. 2 in the down configuration and within 3.3% in the up configuration.

The τ_w patterns observed on the coupon were more complicated than those observed within the duct (Fig. 2). Data are not available for independent verification of the

Fig. 3. Mean fluid detachment parameter (DP) versus mean detachment force (DF; tensile load) for zebra mussels (*D. bugensis*) attached to various substrates. Linear regression (solid line) and 95% confidence limit (dotted lines) are presented along with related statistics. Error bars = SE.



τ_w mappings, but the general features of these maps can be compared with τ_w inside the duct. For example, one would expect the τ_w a short distance downstream of the duct to be similar to that within the duct. This occurred several centimetres downstream in the up configuration, where the local maxima in τ_w were on average within 0.1% of that in the duct. However, in the down configuration, the maximum τ_w were on average 16% larger than in the duct. This difference may be in part due to streamline curvature following impingement, due to the 3° inclination of the duct.

These results indicate that the wall jet provides a reasonably well-defined flow environment in which to measure the bulk properties of adhesion or attachment strength of benthic organisms such as zebra mussels. It is important to note that the present design is limited to small organisms (i.e., <<2 cm in height) that can be detached by a nominal wall shear stress (τ_w) less than 130 Pa. The latter limitation could be overcome by an increase in pumping capacity, while the former would require a larger duct.

We emphasize that τ_w values presented in this study are nominal values, for several reasons. Firstly, τ_w contour maps were made without mussels present; the presence of a mussel would influence the flow pattern and thus change the τ_w distribution. Secondly, τ_w contour maps were determined on a smooth coupon using a Preston tube which had been calibrated for flow over a hydraulically smooth surface. The fact that some of the substrates were rough means that the actual τ_w values would deviate somewhat from the nominal τ_w values. However, because the Preston tube diameter (1 mm) is larger than the viscous sublayer thickness (≤0.2 mm for flow rates of interest), this effect is not expected to be substantial, so long as τ_w is interpreted as the shear stress which would have existed on a smooth surface at that particular flow rate. Ultimately, the validity of using the nominal τ_w to characterize the mussel attachment strength is confirmed by the excellent correlation between DF and DP presented in Fig. 3. If our τ_w values were grossly in error due to the effects described above, we would not expect the strong correlation which was observed.

Zebra mussel attachment strength

The mechanics of mussel detachment are of interest in the data analysis. Preliminary results from adhesion on mild steel (J.D. Ackerman et al., unpublished data) indicate that, under wall jet testing and tensile loading, approximately 40% of the byssal connections failed at the byssal pad – substrate interface and that the majority of the remaining failures occurred within the byssal thread. Although it would be of interest to isolate the actual mechanism of detachment and how these are influenced by the substrate, this was not the goal of this work. Rather, we have simply examined overall detachment strength as a function of substrate type. In doing so by using our wall jet protocol, we assume that mussel detachment is a threshold effect and not due to fatigue caused by previous testing at lower flow rates. For example, a mussel exposed to low flow rates ($<3.06 \times 10^{-3}$ $m^3 \cdot s^{-1}$) remained attached for over 2.5 h. Detachment finally occurred when the flow was increased to 3.06×10^{-3} $m^3 \cdot s^{-1}$. We take this observation as support for the assumption that detachment is indeed a threshold effect.

It is of interest to compare the nominal detachment shear stress values with "critical shear stress" values. The latter quantity is used in the sediment transport literature and

Table 2. Statistical analyses of zebra mussel (*D. bugensis* and *D. polymorpha*) attachment strength on different substrates using the wall jet apparatus and tensile loading tests.

A: Wall jet fluid detachment parameter

Source	df	SS	MS	*F*	*P*
Substrate	4	1.2299	3.075	18.31	<0.001
Error	262	4.3987	0.168		
Total	266	5.6286			

B: Tensile loading test

Kruskal–Wallis	$\chi^2 = 139.46$	df = 4	$P < 0.001$

C: Tensile loading tests between D. bugensis and D. polymorpha

ANOVA	$F = 0.29$	$P = 0.59$
Wilcoxon	$Z = -2.78$	$P = 0.006$

represents shear stress required to move a passive nontethered particle such as a sand grain or rock. It can be estimated using the Shields curve (Dyer and Soulsby 1988). To determine an upper bound on this quantity, we considered a mussel of "diameter" 10 mm and specific gravity 1.1, for which the critical shear stress calculated from the Shields curve was approximately 0.5 Pa. In all cases observed in this study, this is a small fraction of the detachment shear stress. We conclude that the detachment shear stresses were indicative of the strength of byssal attachment, rather than representing a passive resistance to motion.

The attachment strength of zebra mussels varied significantly among substrates under both measurement techniques (Table 2). A single classification analysis of variance (ANOVA) of DP among substrates was highly significant ($P < 0.001$; Table 2), indicating that DP varied among substrates. Multiple *a priori* comparison tests indicated that the inferred attachment strength was significantly different among rocks and the other substrates ($P < 0.001$), PVC and PMMA ($P < 0.001$), and SS and PMMA ($P < 0.01$). The attachment strengths of postlarval mussels were significantly different from those of adult mussels, although significant differences between PVC and SS were not detected. Grouping limestone and dolomite rock samples into a single category ("rocks") was justifiable given that significant differences between these natural substrates were not found ($P = 0.681$).

With regards to the tensile loading experiments, a Kruskal–Wallis test (a nonparametric single classification test) of the DF data among substrates was significant ($P < 0.001$; Table 2), indicating that tensile load varied with substrate. Nonparametric sign tests among substrates revealed that the following pairs of substrates had significantly different attachment strengths ($P < 0.01$): rocks and AL, rocks and PMMA, PVC and AL, PVC and PMMA; SS and PMMA, SS and AL, and PMMA and AL.

A comparison between the two species of zebra mussels was only possible for the tensile loading experiments on rocks. Significant differences were not detected under a single classification ANOVA ($F = 0.29$; $P = 0.59$) but were detected under a Mann–Whitney *U*-test ($Z = -2.78$; $P = 0.006$). The discrepancy between these methods may be due in part to the differences in sample sizes. From our perspective, these two species of zebra mussels are functionally similar in their attachment strength (1.55 ± 0.07 N for *D. bugensis*; 1.4 ± 0.4 N for *D. polymorpha*). Although it should be noted that significant differences in attachment strength have been found among closely related species, as in the case of *Mytilus* species hybrids (Willis and Skibinski 1992), these results are broadly consistent with tensile loadings of other bivalves (10–90 N for an average shell length of 3–3.5 cm; Waite 1983). However, the force required to detach individual zebra mussels from their dense aggregations, in which byssal connections extend among many levels of neighboring individuals, will likely be greater.

These results can be compared with a previous analysis of small (≤2 mm) *D. polymorpha* using a rotating disk test system (Ackerman et al. 1992, 1993). The average nominal detachment τ_w values measured using the rotating disk system were between 30 and 48% of the present results (i.e., DP/l^2; 16.0 ± 0.5, $n = 407$ on AL disks; 20.6 ± 0.6, $n = 472$ on PMMA disks; 22.2 ± 0.8, $n = 335$ on SS disks; 27.7 ± 0.7, $n = 766$ on PVC disks; Table 1). Differences between τ_w measured using the wall jet and the rotating disk were significant (single classification $F >> 3.85$; $P < 0.001$ in all cases). This conclusion was not unexpected given the difference in technique and mussel size. What was unexpected were the differences in ranking of attachment strength on AL and PMMA disks and coupons. This may be due to the health of mussels being affected by observed corrosion of the AL disks, which did not occur with the AL coupons placed in Lake Erie (Ackerman et al. 1992, 1993). The situation with PMMA is less clear, but may reflect differences related to the use of dechlorinated tap water (rotating disk) versus lake water (wall jet). Nevertheless, for a given substrate the measured τ_w were within a factor of 2–3 for both techniques.

There are significant differences in the attachment strength of zebra mussels among substrates. Moreover, there are differences between postlarval and adult mussels. From a practical perspective, a further elucidation of these differences may provide useful design criteria for engineering applications. Ecologically, the attachment strength of individual zebra mussels is comparable with the epibyssate adhesion of marine bivalves. However, the attachment strength of zebra mussels within "thick" aggregations remains to be characterized. Future research should be directed at the mechanism by which detachment occurs, which appears to be a combination of interfacial and byssal thread strength.

Evaluating the wall jet

Detachment fluid forces in the wall jet apparatus were inferred in part from the nominal wall shear stress (τ_w) measured on a flat coupon ($DP = \tau_w \cdot l^2$). These DP values do not include the effects of the organism on the flow,

and for this reason are considered throughout as the "fluid detachment parameter". Although the actual fluid forces on an organism are complex, involving lift, drag, and torque forces (Basmadjian 1984; Hall 1988), we show in the Appendix that the nominal τ_w is approximately proportional to the net detachment force acting on a mussel of a given size.

Including an area factor in the definition of DP should allow the DP to be applied to zebra mussels of different sizes. This is confirmed by our experimental data, which show that the results of the wall jet experiments correspond well with the tensile loading measurements in terms of the ranking of zebra mussel attachment strength on the different substrates. Moreover, Fig. 3 shows that the correlation between DP and DF (tensile load) is high, and the regression of DP on DF is significant ($P < 0.01$) and meaningful ($r^2 = 0.97$). Not unexpectedly, the attachment was strongest on rock samples, which were macroscopically rough compared with the coupon substrates. In addition, the DP–DF correlation would have been stronger if the mussels that could not be removed from rocks under the present wall jet design could have been tested and included in the analysis. Overall, these results confirm that DP provides a reliable inference of attachment strength.

We conclude that the wall jet is a useful and convenient device for making inferences of the attachment strength of benthic organisms, thereby gaining insight to their biology and ecology (Vogel 1981; Denny 1988). If lift and drag forces on zebra mussels and other organisms in a given flow were better understood, it would be possible to calculate the actual detachment forces from wall jet flow rate measurements.

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Appendix: Relationship between wall shear stress and detachment force

In this appendix, we show that nominal wall shear stress values (obtained for flow over a flat coupon) are approximately proportional to the detachment forces acting on a mussel. We draw on the results of Wiberg and Smith (1987), who examined forces on small objects next to a smooth boundary in turbulent flow. They concluded that lift and drag forces on such objects (F_L and F_D (newtons), respectively) are proportional to local fluid velocities as follows:

$$[A1] \quad \begin{aligned} F_D &\propto \bar{u}^2 \\ F_L &\propto (u_T^2 - u_B^2). \end{aligned}$$

In the above expressions, $\bar{u}$ is the average velocity (metres per second) over the height of the mussel given by

$$[A2] \quad \bar{u} = \frac{\int_{z_B}^{z_T} u(z) \cdot dz}{(z_T - z_B)}$$

and z_T and z_B are the elevations (metres) of the top and bottom of the mussel, respectively. Similarly, u_T and u_B are the values of $u(z)$ at z_T and z_B. For turbulent flow over a flat plate, it is well known that the velocity profile satisfies the log law

$$[A3] \quad \frac{u(z)}{u^*} = \frac{1}{\kappa} \ln\left(\frac{z \cdot u^*}{\nu}\right) + b$$

where $u^* = \sqrt{\tau_w/\rho}$ is the friction velocity (metres per second), κ is the von Karman constant (=0.41), and b is a constant (≈5). Using Eq. A3, we can evaluate F_D and F_L for parameter values typical of flow over a zebra mussel. Specifically, Eqs. A2 and A3 give

$$[A4] \quad \bar{u} = \frac{u^*}{\kappa \cdot (z_T - z_B)} \cdot \left[z_T \cdot \ln\left(\frac{z_T \cdot u^*}{\nu}\right) - z_B \cdot \ln\left(\frac{z_B \cdot u^*}{\nu}\right) \right] - \frac{u^*}{\kappa} + b \cdot u^*.$$

Using $z_T = 5.0 \times 10^{-3}$ m, $z_B = 5.0 \times 10^{-4}$ m, and $\nu = 1 \times 10^{-6}$ m$^2 \cdot$s^{-1}, we note that the bracketed term in Eq. A4 varies by only 13% for τ_w values from 20 to 130 Pa (equivalent to a u^* variation of 160%). Thus, to a good approximation, we may assume that $\bar{u}$ is proportional to u^*, so that the drag force F_D is proportional to $(u^*)^2$, i.e., to τ_w.

Similarly, the use of Eqs. A3 and A1 shows that F_L is proportional to

$$[A5] \quad u_T^2 - u_B^2 = \frac{u^{*2}}{\kappa^2} \cdot \ln\left(\frac{z_T}{z_B}\right) \cdot \left[\ln(z_T \cdot z_B) + 2\ln(u^*) - 2\ln(\nu) + 2b \cdot \kappa\right].$$

Once again, the bracketed term is relatively constant, varying by only 13% for τ_w values in the range of 20–130 Pa (equivalent to a u^* variation of 160%). Thus, F_L is approximately proportional to $(u^*)^2$, i.e., to τ_w. Since F_D and F_L are both proportional to τ_w, we conclude that the net force on a mussel, which is simply the vectorial sum of F_D and F_L, is also proportional to the nominal wall shear stress, τ_w.

Finally, it should be pointed out that the derivation presented above has assumed a turbulent velocity profile appropriate for a smooth coupon (Eq. A3). Coupon roughness will alter the constant in Eq. A3 by an amount proportional to $\epsilon \cdot u^*/\nu$, where ϵ is the nominal coupon roughness. However, for the range of interest in the present study, this does not alter our conclusion that the force on a mussel will be proportional to the nominal wall shear stress, τ_w.

Density-dependent survival as a function of size in juvenile salmonids in streams

Elizabeth A. Marschall and Larry B. Crowder

Abstract: The bioenergetic mechanism of density-dependent survival in juvenile stream salmonids was modeled using the relationship between density, food availability, and body size for a food-limited stream system. With a consumption-rate function estimated from laboratory experiments reported in the literature, we developed a function to predict maximum density of stream-resident juvenile salmonids of different sizes. This function can thus predict effective density based on both numbers and sizes of fish. Density predictions from this mechanistic function did not differ from predictions in the literature based on an empirically derived function describing the relationship between minimum territory size and body size in age-0 salmonids in streams, but it did suggest a bioenergetic mechanism for these relationships, linking size and consumption rate to the available food. Finally, we proposed a model of per capita survival rate as a function of the numbers and sizes in a cohort of stream-resident juvenile salmonids.

Résumé : Nous avons modélisé le mécanisme bioénergétique de la survie dépendante de la densité chez des salmonidés juvéniles d'eau courante en utilisant la relation entre la densité, la disponibilité de la nourriture et la taille corporelle pour un système lotique oligotrophe. À l'aide d'une fonction du taux de consommation estimé à partir d'expériences de laboratoire rapportées dans la littérature, nous avons élaboré une fonction permettant de prédire la densité maximale de salmonidés juvéniles d'eau courante de différentes tailles. Cette fonction peut donc prédire la densité effective à partir du nombre de poissons et de leur taille. Les prédictions de la densité à partir de cette fonction mécaniste ne différaient pas des prévisions trouvées dans la littérature et fondées sur une fonction empirique décrivant la relation entre la superficie minimum du territoire et la taille corporelle chez des salmonidés d'âge 0 en rivière, mais elles ont permis de décrire un mécanisme bioénergétique pour ces relations, en liant la taille et le taux de consommation à la nourriture disponible. Enfin, nous proposons un modèle du taux de survie par individu comme fonction du nombre de poissons et des tailles dans une cohorte de salmonidés juvéniles dulcicoles.
[Traduit par la Rédaction]

Survival early in the life history of salmonids in streams has been shown to be dependent on cohort density (LeCren 1965; Chapman 1966; Allen 1969; Elliott 1987, 1989, 1990; Titus 1990). Predicting dynamics of a cohort under different environmental conditions requires understanding the basis of this density dependence. Individual salmonids defend territories in the streams beginning very early in their life history (Latta 1969; LeCren 1972; Noakes 1980; Titus 1990), so space is clearly an important resource. But space itself may be a surrogate for some vital resource available in that space. In salmonids, territories are primarily for feedings, so density of fish in a particular space during the growing season depends on food availability (Mason and Chapman 1965; Slaney and Northcote 1974; Mason 1976; Dill et al. 1981). Because individual consumption rate increases with body size (Elliott 1975), density of fish supported by a particular food resource also will depend on body sizes.

Here, we model the relationship between density of first-year stream salmonids, food availability, and body size. We include a brief description of an analogous function for space-limited stream salmonids, originally derived by Grant and Kramer (1990) with a new parameter estimate from Grant (1993). Finally, we give a function for density-dependent survival of young stream salmonids in a food-limited system.

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Model development

Food limitation

When a food is limited, a given area of stream will be able to support fewer individuals in a cohort of large fish than in a cohort of small fish. The impact of a cohort of

small fish on prey resources will be less than the impact of an equal number of large fish. Assuming that food is limiting, the strength of density dependence will be based not only on the absolute number of fish, but on the effect that the number has on the food resources. We refer to this as the "effective density" or the consumption-potential density of the cohort. Note that when we refer to large fish and small fish, we are only referring to those sizes expressed throughout the first growing season.

Clearly, this effective density will be a function of both the body sizes and number of fish in a cohort. We assume here that a given amount of food will support a given effective density of fish, but that the actual numbers supported will depend on their body sizes. Because daily consumption rate is a function of body size, we can derive a functional link between number and size of fish in a cohort. Under constant environmental conditions, daily food consumptions, C (g dry wt.·d^{-1}) as a function of body size has the form

$$C(m) = \alpha m^{\beta}$$

in most fish (Wooton 1990), where m is mass (g) of an individual. Parameters α and β (see Table 1 for definitions) have been estimated for brown trout (*Salmo trutta*, a stream salmonid) feeding ad libitum at $\alpha = 0.04$ and $\beta = 0.77$ (Elliott 1975). If a habitat has F total available food density per day (g dry wt.·m^{-2}·d^{-1}), and if a density of n_F individuals per square metre with individual body mass m are using ρ_F proportion of this food, then

$$\rho_F F = n_F \alpha m^{\beta}.$$

So $n_F \alpha m^{\beta}$ represents the effective density, relative to food available, of this group of fish. Clearly, as body mass m increases, numbers n must decrease to maintain an equivalent impact on the food resources (i.e., an equivalent effective density):

$$[1] \quad n_F = \rho_F F \frac{1}{\alpha} m^{-\beta}.$$

Note that α and β have been estimated independently of this function for brown trout (Elliott 1975), but that $\rho_F F$ (total amount of available food used) often will be much more difficult to estimate.

Space limitation

Grant and Kramer (1990) empirically estimated an analogous function for space limitation in stream salmonids. In a review of the literature, they found 23 studies in which minimum territory size (cm^2) and fish sizes were both measured. They regressed log(1/territory size) on log(fish size) to obtain space-limited density (n_S) as a function of fish size:

$$n_S = \rho_S \gamma \ell^{-\omega}$$

where ω and γ are both estimated by regression, ρ_S is the proportion of total habitat used, and ℓ is fish total length (cm). Their parameters were estimated by regression under the assumption that all of the available habitat was used, i.e., $\rho_S = 1$ (because data used in the regression are minimum territory sizes when habitat is at saturation, Grant and Kramer 1990).

Table 1. Summary of definition of parameters used in the text.

Parameter	Definition
C	Individual daily food consumption (g dry wt.·d^{-1})
m	Individual body mass (g)
FL	Fork length (cm)
ℓ	Total length (cm)
F	Total daily available food density (g dry wt.·m^{-1}·d^{-1})
ρ_F	Proportion of total food used in food limitation model
ρ_S	Proportion of total space used in space limitation model
n_F	Density of age-0 fish in food limitation model
n_S	Density of age-0 fish in space limitation model
α	Intercept of consumption versus mass relationship (estimated from Elliott 1975)
β	Exponent of consumption versus mass relationship (estimated from Elliott 1975)
γ	Intercept of density versus length relationship, best-fit estimate
ω	Exponent of density versus length relationship, best-fit estimate
D	Effective density, a sum of number of fish times their potential consumption

Model comparison

To compare the food limitation model with the Grant and Kramer (1990) model, we described both as a functions of total length, rather than mass. We used the relationships

$$m = 85.11\ (FL)^{3.03}$$

(Grant and Kramer 1990) and

$$FL = 0.9684\ell$$

(Carlander 1969) to give a relationship to convert between ℓ (total length in cm) and m (mass in g):

$$m = 77.23\ell^{3.03}.$$

Then our original function describing n_F as a function of m (eq. 1) can be rewritten as function of ℓ (where $\alpha = 0.04$, $\beta = 0.77$, Elliott 1975)

$$n_F = \rho_F F \frac{1}{\alpha} (77.23\ell^{3.03})^{-\beta} = \rho_F F \cdot 0.8798\ell^{-2.33}.$$

We also assumed $\rho_F = 1$, meaning the population is at the level of saturation of the food resource, to make it comparable to Grant and Kramer's estimate of ω and γ assuming $\rho_S = 1$.

Although we have estimates for α and β (from Elliott 1975), we must estimate F to be able to make comparisons between the two functions. We can estimate F from a log-log regression of density of fish on total length:

$$\log(n_F) = \log(0.8798F) - 2.33 \log(\ell).$$

To estimate the one remaining parameter, we used the same data from 23 studies that Grant and Kramer used in

their estimate of γ and ω (see Table 1, Grant and Kramer 1990). However, because we were only interested in the relationship during the first part of the first growing season for a given cohort (the time during which year-class strength is set in many cases, Elliott 1989), we limited the data to those cohorts made up of fish less than 8 cm (resulting in 18 observations). We found

$$n_F = 506\ell^{-2.33}.$$

Equation 2, for a space-limited system with both ω and γ as estimated from regression on these same data, is

[2] $$n_S = 477\ell^{-2.30}.$$

Grant (1993) reevaluated the relationship and concluded that a model II regression would have been more appropriate (Sokal and Rohlf 1981). We re-estimated the parameters using a model II regression on the same data set to find

$$n = 587\ell^{-2.57}.$$

The slope in our food-limitation model, estimated independently of the territory size data, does not differ significantly from the slope of the least squares regression through these data (T-test, $p = 0.93$) nor the model II regression through these data (T-test using standard error from model I regression (Sokal and Rohlf), $p = 0.57$). The food limitation model, based on independent, a priori estimates of both slope and the form of the function and only using the data to estimate the intercept, or location of the function, fit the data almost identically to the function that was the best-fit line through those data. The two models are nearly equivalent in their predictions of maximum density as a function of body size in stream salmonids in their first growing season.

Grant and Kramer (1990) compared their model with independent data from the literature. Their model performed remarkably well in describing the effective density limit above which a cohort will exhibit density-dependent mortality, emigration, or growth. Our model suggests a bioenergetic basis for Grant and Kramer's (1990) empirical result.

Density-dependent survival rate

Above, we derived an expression of effective density of a cohort as a function of sizes of individuals in the cohort and used data compiled by Grant and Kramer (1990) to estimate the parameters of the function. We next suggest a way to use these relationships to describe density-dependent survival in young-of-the-year stream salmonids. Note that when we use the term "survival" here, we are really speaking about "local survival," that is, the proportion of fish that both survive and do not emigrate. Both mortality and emigration are possible responses to density in young stream salmonids (Elliott 1987; Titus 1990). To develop a relationship between survival rates of individual stream salmonids and effective density requires data that include sizes of fish in a cohort and numbers of fish at two sample times. At any sample time t, the effective density, $D(t)$, of a cohort (g dry wt.·m^{-2}·d^{-1}), is the sum of the number of fish in each size class times their size-dependent potential consumption

$$D(t) = \sum_i n_i(t)\,\alpha m_i^{\beta}$$

where m_i is the mass (g) of fish in size class i and $n_i(t)$ is the number of fish in that size class at time t.

Per capita survival rate, $r(t)$, of fish in this cohort at time t, should increase with decreasing effective density. Clearly, survival rate cannot increase beyond one, but we believe the maximum will be below one. With decreasing effective density, the population may become limited by something other than food availability. Here, we have assumed that at low effective densities, per capita survival becomes density independent, taking a value c_0, an estimate of density-independent survival. At high effective densities, per capita survival rate will approach zero:

$$r(t) = c_0 D(t)^{-c_1}.$$

Discussion

Our goal in deriving a relationship between size of fish and density of fish for age-0 stream salmonids was to be able to represent the interplay between size and density in their effect on survival rates. In modeling the early life history of stream salmonids, we are faced with the dilemma of knowing that local survival rates are density dependent (LeCren 1965; Chapman 1966; Allen 1969; Elliott 1987, 1989, 1990; Titus 1990), but of not having an explicit way to represent this dynamically in a model. It is obvious that we should not model density-dependent survival as a constant number of fish over time when fish are changing sizes over time. Others have proposed that density dependence may be expressed dynamically (i.e., over time as fish are growing) as a constant biomass of fish being supported in a given area, but there is really no mechanistic basis for this assumption and Grant (1993) has recently provided evidence against it. In choosing to model survival in this early life stage as a function of bioenergetic demand on the food resources, we have a logical basis for our assumptions (e.g., Egglishaw 1967; Symons 1971; Dill et al. 1981; Wilzbach 1985) and we can easily include it as a relationship that is dynamic as fish change sizes. Thus, we have derived a representation of density-dependent survival that is (*i*) flexible over size changes of fish early in their first growing season, (*ii*) mechanistically based on a justifiable assumption (bioenergetic demand), and (*iii*) agrees with empirical measures of size and density relationships (comparison with data from Grant and Kramer 1990). This is an important step in our ability to model population dynamics of stream salmonids (Marschall 1991).

Other investigators have also proposed models relating numbers of animals in a given area to body size. Our food-limitation model is similar to Begon's −4/3 thinning rule for animal populations, which he based on metabolic rate scaling by a 0.75 exponent of mass (Begon et al. 1986; Elliott 1993). Although Peters (1983) calculated different relationships for different types of animals, he collected estimates of the exponent of the metabolic rate versus body size relationship for fishes in general (0.78 and 0.81, Winberg 1960), teleosts (0.70, Kayser and Heusner 1964; 0.81 and 0.77, Zotin and Konoplev 1978), salmonids (0.76 ± 0.032, Winberg 1960), and salmon (0.775, Brett 1965).

These values are similar to the exponent used in the model presented here (0.77, Elliott 1975), and thus would yield similar predictions.

Grant and Kramer's (1990) space-limitation model is based on the assumption that individual salmonids require a minimum amount of space (as a function of body size) in a stream, and that there is a constant amount of usable area per area of stream. Our food-limitation model is based on the assumption that an individual salmonid has a minimum requirement of food that scales by a 0.77 exponent of its body mass and that there is a constant amount of food available per area of stream. In general applications of these models, the assumption of constant usable area or constant food available can be interpreted as some expectation of the amount of usable area or food per area of stream, rather than as a strictly invariable resource density.

As well as being useful in modeling early life history of stream salmonids, derivation of this relationship has proven useful in designing experiments involving fish of different sizes. For example, in an experiment to look at growth and survival effects of pH level on two sizes of brook trout in constructed stream channels with a natural food source, we wanted to equalize the effect that food abundance would have on survival in each of the two sizes of fish (Marschall 1991). This would be necessary to accurately describe a null hypothesis in any experiment investigating size dependence of survival responses. To do this, we equalized the effective density, or the consumption-potential density, between the two size classes in the experiments. In this way, we could attribute size-dependent differences in growth and survival to size-dependent differences in response to the treatment rather than to differential limitation by the food resources.

It is well established that local survival of age-0 stream salmonids is density dependent. It is also well established that body size plays an important role in many aspects of salmonid life histories (e.g., probability of survival, fecundity, ability to hold feeding territories). To expect to wholly understand the dynamics of stream salmonid populations, we must be able to understand the interplay of size and numbers, of how available resources might support different numbers of different sized individuals, and how different sized individuals will differentially impact the resources available to other individuals. This will require eventual focus on each of many different life stages and processes. As a first step in this direction, our model suggests a way to mechanistically link density-dependent survival in age-0 fish to food availability; "effective density" integrates the effects of body size and number in characterizing the bioenergetic demand of a young cohort on their prey resources.

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Bioenergetic analysis of prey consumption by *Bythotrephes cederstroemi* in Lake Michigan

Peder M. Yurista and Kimberly L. Schulz

Abstract: A bioenergetic model for *Bythotrephes cederstroemi* was constructed using measured physiological parameters to predict predation rates. The model predicts that juvenile *B. cederstroemi* will consume approximately 150% of their body weight per day, while adults consume 118% of their body weight per day. These rates are consistent with those of other invertebrate crustaceans. The predicted rate was twice that of an experimental measurement reported for Lake Huron *B. cederstroemi*; this discrepancy is attributed to experimental artifacts and to differences between *B. cederstroemi* populations in Lake Michigan and those in Lakes Huron and Erie. The model was most sensitive to estimation of ingestion and assimilation efficiencies and, secondarily, respiration coefficients. This model estimates the consumption rate of *B. cederstroemi* in Lake Michigan under optimal food resource conditions, and may be useful in predicting the future impact of *B. cederstroemi* predation on the zooplankton assemblages of other lakes.

Résumé : Nous avons construit un modèle bioénergétique de *Bythotrephes cederstroemi* en utilisant des paramètres physiologiques mesurés pour prédire les taux de prédation. Le modèle prédit que les juvéniles de *B. cederstroemi* vont consommer un poids de nourriture correspondant à environ 150% de leur poids corporel par jour, contre 118% chez les adultes. Ces taux sont comparables à ceux qu'on a mesurés chez d'autres crustacés. Le taux prédit était le double d'une mesure expérimentale signalée pour *B. cederstroemi* du lac Huron; cet écart est attribué à des artefacts expérimentaux et aux différences entre les populations de *B. cederstroemi* du lac Michigan et celles des lacs Huron et Érié. Le modèle était surtout sensible aux estimations de l'efficacité de l'ingestion et de l'assimilation puis, de façon secondaire, des coefficients de respiration. Ce modèle estime le taux de consommation de *B. cederstroemi* du lac Michigan dans des conditions trophiques optimales, et peut être utile pour prédire l'impact futur de la prédation exercée par *B. cederstroemi* sur les assemblages zooplanctoniques d'autres lacs.
[Traduit par la Rédaction]

Introduction

Energy transfer has long been investigated as a common link between ecosystem components (Lindeman 1942). Bioenergetic budgets and models of this energy transfer serve to synthesize existing physiological data for a population and to make forecasts of the consequences of this data. These models have been used extensively in fisheries research (Kitchell et al. 1977; Brett and Groves 1979; Kitchell 1983; Rice and Cochran 1984; Stewart and Binkowski 1986; Adams and Breck 1990), but less often in zooplankton studies. Research on the herbivore *Daphnia* has provided a great wealth of information on feeding, assimilation, respiration, food selection, growth, and reproduction that has been the basis of several bioenergetic models for *Daphnia* populations (Paloheimo et al. 1982; Kooijman 1986; McCauley et al. 1990). However, such models have not been used for other herbivorous zooplankton, nor have the bioenergetic requirements of most invertebrate predators been well studied. The several models that do exist include those for the cold-water predator *Mysis* (Rudstam 1989) and the copepod *Megacyclops* (Krylov 1988).

The scarcity of bioenergetic models for invertebrate predators leaves a gap between the *Daphnia* and fish models that limits understanding of aquatic food webs. Invertebrate predators can be important in structuring planktonic communities, and several authors have investigated the hypothesis that in the absence of fish, predation by invertebrates will shift the size structure of the zooplankton community toward larger-bodied animals (Sprules 1972; Dodson 1974; Vanni 1988). However, the magnitude of predation by invertebrates in systems that include fish remains poorly understood.

The recent introduction to the Laurentian Great Lakes of *Bythotrephes cederstroemi*, a large-bodied predatory cladoceran (Bur et al. 1986; Lange and Cap 1986; Lehman 1987; Cullis and Johnson 1988), has been correlated with community food web changes (Lehman 1991). This perturbation to the pelagic community redefines the structure

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and intensity of biological interactions, and presumably alters energy flow within the system. For example, zooplankton that are prey for young-of-the-year fish are now potentially a shared resource with *B. cederstroemi*. Quantifying the effects of these changes is important for interpreting the response of food webs in the Great Lakes. The magnitude of predation by *B. cederstroemi* in the Great Lakes is controversial (Sprules et al. 1990; Lehman 1991), and little documentation on *B. cederstroemi* feeding behavior exists (Mordukhai-Boltovskaya 1958; Vanderploeg et al. 1993) that would help to resolve the question of whether predation by *B. cederstroemi* has the potential to impact food webs in the Laurentian Great Lakes. Furthermore, the continued spread of *B. cederstroemi* to other waters is inevitable (Yan et al. 1992) and of concern to wildlife managers.

The ability to assess energy loss from the system through a bioenergetics approach will facilitate estimation of the energy available for biomass production within the food web. The energy balance of *B. cederstroemi*, as in all organisms, depends on energy consumption and loss processes. Very little basic biology or metabolic physiology of *B. cederstroemi* exists in the literature (see Yurista 1992). The purpose of this study was to use our recently measured values of ingestion, assimilation, and respiration for quantifying energy flow in *B. cederstroemi* from Lake Michigan. A bioenergetic simulation was used to predict predation rates and these results were compared with previously published work on *B. cederstroemi* in the Laurentian Great Lakes.

Methods

The *B. cederstroemi* bioenergetic model was based on six processes: (1) consumption, (2) ingestion, (3) assimilation, (4) respiration, (5) exoskeletal molting, and (6) growth and reproduction. The energy allocation to growth and reproduction was subdivided into three compartments representing the three instars to primaparity (Yurista 1992). A pair of lateral barbs is added to the tail spine at each instar; a mature animal possesses three barb pairs. *Bythotrephes cederstroemi* population structure can be assessed readily from samples collected in the field by using the number of barb pairs to identify the instar of individuals. Because all three instars are predatory, this quick identification method facilitated investigation of the total impact of the *B. cederstroemi* population on the food web. *Bythotrephes cederstroemi* reproduces parthenogenically throughout much of the summer growing season. Therefore, modeling female growth and reproduction is a reasonable representation for the majority of the population. Garton and Berg (1990) found the maximum frequency of males to be 4% in Lake Erie and 42% in Lake Superior. If a population contained substantial numbers of males, these individuals could be included in the model as animals that progress through only the first and second instars. Males typically do not add a third pair of barbs to their tail spines and remain similar or slightly larger in mass than female second instar animals (Garton and Berg 1990; Yurista 1992). If males were abundant, separate growth curves for males could be constructed (see below). We assume the following bioenergetic relationship:

$$[1]\quad \text{Growth and reproduction}(i) = \text{consumption}(i) \times \text{ingestion efficiency} \times \text{assimilation efficiency} - \text{respiration}(i) - \text{molt}(i) \qquad i = 1,2,3 \text{ instar.}$$

Bythotrephes cederstroemi were collected from offshore Lake Michigan on several cruises during the summer of 1991 with a 1-m-diameter net having 300-μm mesh size and a solid 2-L PVC cod end. As the phototaxic animals swam to the surface of the cod end, they were sorted by hand using jeweler's forceps to grasp them by their tail spines. *Bythotrephes cederstroemi* were placed into individual 2-mL tissue culture wells filled with glass fiber (Whatman GF/F) filtered Lake Michigan water and fed *Artemia* nauplii (Yurista 1992). These animals were allowed to acclimate to laboratory conditions for at least 24 h at 16°C prior to all subsequent measurements. All parameters were estimated at 16°C.

Ingestion and assimilation

Uniformly radiolabeled *Daphnia pulicaria* were used to estimate ingestion and assimilation of individual prey by *B. cederstroemi*. *Daphnia pulicaria* were labeled with either ^{14}C or ^{33}P by feeding on uniformly labeled *Chlamydomonas reinhardi* for at least 72 h.

To measure ingestion, ^{14}C-labeled *D. pulicaria* were grasped by the base of the tail spine with fine-tipped forceps and offered to *B. cederstroemi* in tissue culture wells. *Daphnia pulicaria* handled in this fashion were grabbed readily and consumed by *B. cederstroemi*. The percentage of each prey item ingested was calculated as the percentage of radioactivity present in the *B. cederstroemi* relative to the total amount of radioactivity in both the *B. cederstroemi* and the debris remaining in the tissue culture well after feeding. Only the 71% of trials in which *B. cederstroemi* fed well (defined as shredding the whole prey item, not simply dropping or mangling it) were included when calculating the percentage ingested for this model. This is a conservative value for calculating the amount of carbon required for *B. cederstroemi* to grow, as it is a maximal estimate of ingestion efficiency. A lower estimate, which included prey that were damaged or partially consumed, would require that more prey be killed (a higher predation rate) to achieve the same net carbon intake.

Assimilation was measured using ^{33}P-labeled *D. pulicaria*. The *D. pulicaria* were fed to the *B. cederstroemi* as before, but after feeding was completed, the *B. cederstroemi* were removed from their cell wells and incubated for 1, 2, 4, 6, or 12 h in scintillation vials filled with 3 mL of GF/F-filtered Lake Michigan water. *Artemia* were added to the incubation vials to allow *ad lib* feeding. The percentage assimilated of what was ingested was calculated as the percentage of the total amount of radioactivity ingested remaining in the *B. cederstroemi* after incubation. A time course of this assimilation value showed a decrease in the rate of ^{33}P release after 6 h. This corresponds to a gut passage time of 6–12 h estimated by following the passage of orange *Artemia* through the digestive tract of *B. cederstroemi*. From these measurements, assimilation was calculated to be 50–60% of ingestion. Again, the higher value was used as a conservative measure of total carbon intake. This assimilation value includes some short-term metabolic excretion; long-term excretory losses of dissolved

organic carbon (DOC) are assumed to be small but have not been measured for zooplankton (Omori and Ikeda 1984).

Respiration

Oxygen measurements were made with a polarographic microprobe (Hansatech; Delieu and Walker 1972). The closed system reaction chamber was surrounded by a water jacket supplied with water from a thermally regulated bath. Values for oxygen concentration in air-saturated water were those from Mortimer (1981). Background consumption of oxygen by the probe was measured three times each day. The oxygen consumption rate for each *B. cederstroemi* was measured in 1.5 mL of filtered (1.2 μm) Lake Michigan water. Light was maintained at low intensity ($<5\mu E \cdot m^{-2} \cdot s^{-1}$) to mimic natural conditions, and to minimize any photo-induced behavioral responses. Each measurement was initiated at 100% oxygen saturation and terminated after 10–20 minutes. Final concentrations were >90% saturation. Consumption rate was determined as the difference between the slope of the respiration curve (total micrograms of oxygen per minute) and the background oxygen consumption of the probe.

Bythotrephes cederstroemi were removed from the measurement vessel and quickly frozen on dry ice at the completion of each trial. The animals were later freeze-dried and weighed to the nearest tenth of a microgram on an electrobalance (Cahn) for determination of weight-related respiration. Approximately 50 measurements were made with animals equally distributed among instars. The relationship of respiration rate to weight was analyzed using nonlinear regression techniques (Wilkinson 1990) to determine parameter values of the allometric equation

$$[2] \quad R = aW^b$$

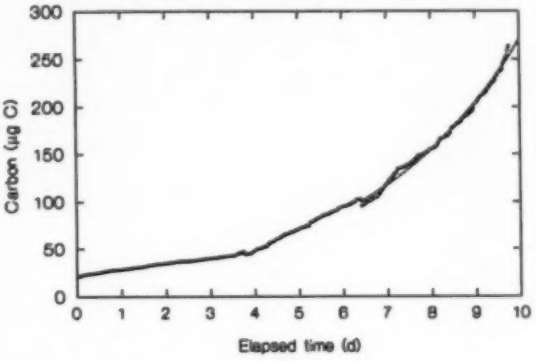
where R is the consumption rate of oxygen (micrograms of oxygen per hour), W is the dry weight of the animals (micrograms), and a and b are dimensionless parameters. Multiple nonlinear regression algorithms (Quasi-Newton and Simplex) were used and generated the same estimates. The respiration rate was converted to carbon equivalents using an RQ of 0.949 for ammoniotelic animals (Lampert 1984). *Bythotrephes cederstroemi* used in respiration calculations appeared healthy and had been feeding on *ad lib* food prior to measurement. Because food concentration is known to have an effect on respiration rate in zooplankton (LaRow et al. 1975), these values are applicable to well-fed animals.

Growth, reproduction, and exoskeletal molting

Growth rates for *B. cederstroemi* are not known because of the difficulty in culturing these animals in the laboratory (Mordukhai-Boltovskaya 1957; Yurista 1992). Therefore, growth rates were estimated from representative samples of animals collected from Lake Michigan. Animals were freeze-dried and weighed on an electrobalance. Dry weight was converted to carbon using a 50% conversion factor (Salonen et al. 1976; Makarewicz and Likens 1979). The animals were ordered by weight within each instar. A total of 542 animals were weighed (163 one-barbs, 113 two-barbs, and 266 three-barbs).

We assumed that this sampling of animals represented the range of weights expected in each instar up to primaparity

Fig. 1. Empirically determined growth trajectory for *B. cederstroemi*. The measured weights (μg C) are shown in ascending order according to their estimated development times at 16°C. Lines fitted to weight data points depict growth curves for each of the three instars.



in Lake Michigan *B. cederstroemi*. This range potentially includes abnormally large and small animals that molted at weights above or below average. To make our growth equations follow the trajectory of the average individual, the difference in weight between the endpoints of the two instars was made to equal the loss due to molting. To accomplish this, equal percentages of animals were truncated from the upper end of the one-barb instar range and the lower end of the two-barb instar until the initial weight of a two-barb individual equalled the final weight of a one-barb individual minus weight loss during molting. This allowed a balanced carbon budget at the time of molt. The loss in molt was taken to be 7% of the animal's weight, which is representative of cladocerans (McCauley et al. 1990). Lynch et al. (1986) determined an allometric function for molt weight in *Daphnia pulex* that ranged from 1.5 to 14% in the animals they measured. Seven percent should be an overestimate for *B. cederstroemi* because their exoskeleton is not very robust (personal observation). The characteristic large tail spine of *B. cederstroemi* is retained during the molt (Yurista 1992), and therefore, the energy investment in this structure is not lost.

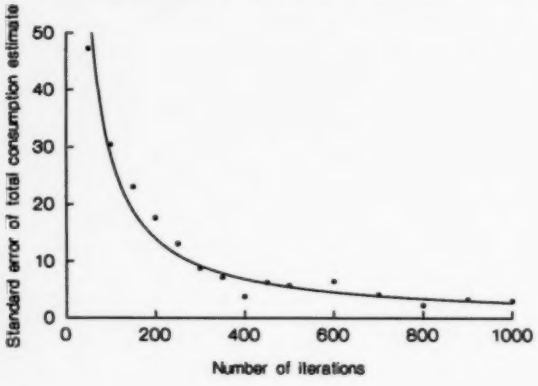
The weight range from the truncation cutoff to the largest animal was then assumed to represent the variability in weight at molting. This variation was assumed to be the same as variation in birth weight, and so the same percentage of animals was truncated from the one-barb instar lower end as had been truncated from the upper end. Because the truncated weights represented the one-sided full range of assumed possibilities at instar shifts, they provided a measure of the variance. This range was assumed to represent 3 standard deviations (where ±3 SD includes 99.7% of a standard normal distribution). A similar process was used at the end of the two-barb and beginning of the three-barb instar distributions.

The time animals spent in each instar was estimated from temperature-dependent development rates in Yurista (1992). The animals were assumed to progress evenly in development and growth throughout each instar until molting. Each weight data point within an instar was assigned

Table 1. Parameters used in the bioenergetics model and their values.

Parameter	Mean (SD)	Units
1. Assimilation	0.60 (0.07)	None
2. Ingestion	0.62 (0.18)	None
3. Time to first molt	3.76 (0.17)	d
4. Time between first and second molts	2.64 (0.17)	d
5. Time between second molt and primaparity	3.37 (0.17)	d
6. Initial neonate weight	22.1 (2.2)	μg C
7. Initial 2 barb weight	42.5 (3.5)	μg C
8. Initial 3 barb weight	99.7 (14.4)	μg C
9. Primaparity weight	254.0 (42.4)	μg C
10. First molt exoskeleton weight	3.1 (0.19)	μg C
11. Second molt exoskeleton weight	7.3 (0.44)	μg C
12. Respiration parameter, *a*	0.088 (0.023)	None
13. Respiration parameter, *b*	0.600 (0.047)	None

Fig. 2. Effect of increasing the number of iterations on the standard error of the total lifetime consumption estimate. An exponential curve is fitted through the data. Five hundred iterations were used in all model estimates.



a development time in proportion to its rank ordered position (Fig. 1). Linear equations were fit to the ordered one-barb and two-barb instar weights, and an exponential equation was fit to the ordered three-barb instar weights. These equations, the initial instar weights, and standard deviations for each instar represent the growth curve used for the model.

The energy investment in reproduction is included in the three-barb growth equation. *Bythotrephes cederstroemi* carry their embryos through development in a large brood sac. Much of the weight increase of three-barb *B. cederstroemi* is attributable to the growth of the embryos (personal observation).

Model

The model was solved for the consumption rate necessary to balance the energy devoted to growth, respiration, molt losses, and the efficiency of conversion of prey into usable carbon:

$$[3]\quad \text{Consumption}(t) = \frac{(\text{growth}(t) + \text{respiration}(t) + \text{molt})}{(\text{ingestion efficiency} \times \text{assimilation efficiency})}$$

where

$$[4]\quad \text{Respiration}_i(t) = (aW_i(t)^b dt)$$

$$[5]\quad \text{Growth}_i(t) = \sum W_i(t) - \text{neonate weight}$$

$$[6]\quad W_1(t) = 22.094 + 6.077\ (t)$$

$$[7]\quad W_2(t) = 42.45 + 23.265\ (t)$$

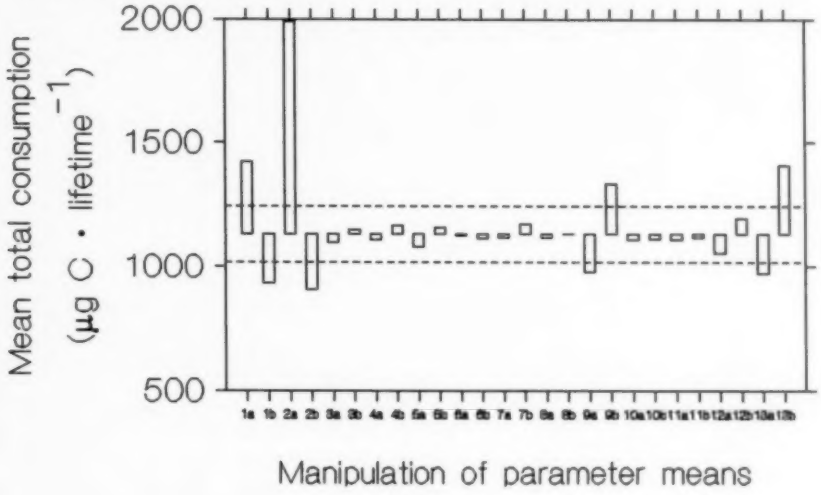
$$[8]\quad W_3(t) = 99.71(10)^{0.1203(t)}.$$

Each instar was modeled as a separate compartment and consumption from all instars was summed for the total consumption to primaparity. All relationships were expressed in micrograms of carbon. Measured parameters are shown in Table 1.

To estimate the error associated with the predicted mean consumption rates, the model was implemented using a Monte Carlo technique; the model was run in compiled QBASIC on a 486 IBM-compatible computer. The random number generator used was a combination of three linear congruential generators, and known to have a long cycle (2.78×10^{13}) along with other desirable properties (Wichmann and Hill 1982; Maindonald 1984). Each parameter was selected randomly from a normal distribution with the measured means and standard deviations as in Table 1. Values generated that did not satisfy biological constraints were rejected and resampled (e.g., $0 \leq$ assimilation < 1.0). The correlated parameters (a, b) for respiration (correlation factor of -0.95) were generated using the technique of Wold (1948).

The number of iterations used to estimate each consumption rate was determined empirically. A series of runs was conducted at intervals from 50 to 1000 iterations. Each run produced an estimated mean consumption rate. This procedure was repeated 50 times, from which a standard error of the mean was calculated. It was determined that 50 simulations of 500 iterations was well past the point of stabilization in the standard error of the mean

Fig. 3. Sensitivity of the mean total lifetime consumption estimate to changes in the means of the parameter estimates. The bars indicate how much the mean total lifetime estimate was perturbed from the standard prediction as a result of changing each mean parameter value ±20% from its measured value. Broken horizontal lines indicate 10% deviations from the mean (1132 μg C). Parameter numbers are as defined in Table 1. The suffix "a" indicates a 20% decrease in the mean parameter value, while the suffix "b" refers to a 20% increase.


Mean total consumption
(μg C • lifetime^{-1})
2000
1500
1000
500
1a 1b 2a 2b 3a 3b 4a 4b 5a 5b 6a 6b 7a 7b 8a 8b 9a 9b 10a 10b 11a 11b 12a 12b 13a 13b
Manipulation of parameter means

(Fig. 2). Therefore, this procedure was used to estimate consumption rates in all runs, including those for sensitivity analysis. All parameters remained constant throughout a given run.

To determine how dependent predicted consumption rates are on the measured parameter estimates, sensitivity of the model to precision of the means of the measured parameters and their variabilities was tested. Each parameter mean or standard deviation was changed by ±20%. The model was then run using the Monte Carlo method. All other parameters were allowed to vary about their originally determined mean values within the Normal distribution defined by their standard deviations. A single parameter or standard deviation was changed for each sensitivity run. All runs were performed twice, once with a fixed seed to the random number generator and once with a randomized seed. Those parameters to which the model was most sensitive were covaried ±10% in additional runs.

Results

The predation rate for *B. cederstroemi* was found to be 48 ± 0.68 (SE) μg C·d^{-1} for one-barbs, 122 ± 2.09 (SE) μg C·d^{-1} for two-barbs, 187 ± 2.74 (SE) μg C·d^{-1} for three-barbs, and 1132 ± 16.94 (SE) μg C total consumption to primaparity. The coefficient of variation was less than 2% for each mean estimate.

Using randomized seeds to initiate each simulation, the sensitivity of the model to changes in mean parameter values was found to be greatest for assimilation, ingestion, and the respiration coefficients (Fig. 3). The largest change observed was associated with decreasing the mean ingestion efficiency by 20%, which resulted in a 75% increased consumption rate above that of the unaltered mean estimate. Increasing the ingestion efficiency by 20% reduced the mean consumption estimate by 20%, indicating a bias in sensitivity to the direction of change. The assimilation parameter produced a 25% higher mean consumption rate with a 20% decrease in the parameter value, and an 18% lower mean consumption rate with a 20% increase in the parameter value. Again, sensitivity was biased to the direction of the change, but the magnitude was more strongly proportional to that of the change in parameter value. While lowering or raising the mean respiration parameter *b* produced a 25% consumption rate decrease or a 38% increase, respectively, in the first instar, the succeeding instars were less sensitive to these changes. The adult instar's consumption rate was the least sensitive to changes in the respiration parameter *b* (14% decrease; 18% increase). Consumption rates were less sensitive to 20% changes in the respiration parameter *a* (13%), but followed the same pattern of instar sensitivity. Consumption rates for each instar were sensitive to 20% perturbations to the growth parameters for that instar (generally 5–10%), while the only growth parameter that affected the total lifetime consumption was primaparity weight (14% decrease; 18% increase). Sensitivity analysis of the model produced similar results using a fixed initial seed.

Changing the standard deviations of the parameters had little effect on the consumption rate. For all parameters with the exception of ingestion, increasing or decreasing the standard deviation by 20% resulted in less than a 5% change in consumption rate. Increasing the standard deviation of ingestion resulted in a 14% increase in predicted

Table 2. Number of each species required to fulfill the daily consumption needs of *B. cederstroemi*. Median lengths and corresponding weights of prey species taken from Bottrell et al. (1976) or Culver et al. (1985).

Prey species	First instar	Second instar	Third instar
Asplanchna	198	504	773
Bosmina longirostris	66	167	257
Calanoid nauplii	407	1034	1585
Ceriodaphnia lacustris	122	309	473
Chydorus sphaericus	94	240	368
Conochilus unicomis	2233	5674	8698
Cyclopoid nauplii	410	1043	1598
Cyclops bicuspidatus	37	93	143
Cyclops vernalis	33	84	129
Daphnia galeata mendotae	8	20	30
Daphnia retrocurva	18	45	69
Diaphanosoma leuchtenbergianum	47	119	183
Diaptomus copepodites	69	174	267
Diaptomus minutus	39	100	153
Diaptomus oregonensis	17	44	67
Diaptomus siciloides	13	32	49
Eubosmina coregoni	36	91	139
Keratella cochlearis	1333	3389	5194
Leptodora kindtii	4	9	14
Mesocyclops edax	30	75	116
Polyarthra vulgaris	2233	5674	8698

consumption rate, while decreasing the standard deviation resulted in a 7% decrease. The model was again most sensitive to variation in the ingestion parameter.

Covarying the ingestion and assimilation parameters by 10% each resulted in an average change of 14% in predicted mean consumption rate (range 1.7–28%). When the ingestion, assimilation, and respiration parameters were all varied simultaneously, the consumption estimate changed an average of 25% (range 12–43%).

The predation rates in micrograms of carbon per day were converted to numbers of average-sized prey consumed using length–weight regressions for Great Lakes zooplankton from Culver et al. (1985). The predation rate in micrograms of carbon per day divided by the weight in carbon of average-sized prey indicates the number of that prey species required to balance the bioenergetic equations (Table 2). A generalist diet would reduce the impact of *B. cederstroemi* on any one specific prey species.

Discussion

The predicted consumption rates are most sensitive to variation in the estimations of ingestion and assimilation efficiencies, followed next by the respiration coefficients, and lastly by development parameters. Ingestion and assimilation parameters affect energy consumption in a multiplicative manner. They were modeled to remain unchanged throughout the animal's life cycle because we found experimentally that ingestion and assimilation are approximately constant for all instars (K.L. Schulz, unpublished data). Sensitivity analysis indicates that ingestion and assimilation are the major controlling parameters in the determination of lifetime consumption by *B. cederstroemi*. The model was next most sensitive to variation in respiration parameters. Respiration coefficients affect energy usage in a nonlinear fashion throughout all life stages. Due to the allometric respiration function, larger animals respire less per unit body mass. Changes in development times, size at instar shifts, and size at primaparity affect the model in an additive fashion, and for only a portion of the range of growth. Altering the time between molts affects only the adjacent instars. The model was least sensitive to variation in these parameters, which did not change the predicted consumption by more than a few percent. Several of these parameters, such as respiration and development time, are temperature dependent. Because these estimates are based on measurements taken at 16°C, and because biological processes frequently are dependent nonlinearly on temperature, the predictions are most applicable when temperatures approximate 16°C. For systems that differ significantly in temperature from 16°C, these temperature-dependent biological parameters should be reestimated and the model rerun if necessary.

The average adult *B. cederstroemi* (355 μg dry wt.; this study) respires 14.4% of its body weight per day. The average one-barb stage (65 μg dry wt.) and two-barb stage (142 μg dry wt.) organisms respire 28.4 and 20.8%, respectively, of their body weight daily. Lampert (1984) reported that the average respiration of starving crustacean zooplankton is 19.1 ± 4.8% of their body weight per day (n = 20). Krylov (1988) reported that an individual of *Megacyclops* must consume 18.2% of its body weight per day to meet respiration needs. During times of stress from intense competition and reduced food intake, copepods are able to metabolize at reduced rates (Comita 1968). In times of stress, large *B. cederstroemi* would be at an advantage because large individuals have a reduced respiratory demand.

Bythotrephes cederstroemi invests a large amount of resources in its offspring. Animals containing several embryos weighed as much as 400 μg C (800 μg dry wt.). After giving birth, these animals weighed less than 175 μg C (350 μg dry wt.). Investment in offspring often represents significantly greater than 100% of the animal's barren weight. For comparison, individual *Daphnia* can invest up to 95% of their net weight gain subsequent to primaparity in reproduction (Taylor 1985). Cladocerans in general have life histories geared toward high reproductive potential (Allan and Goulden 1980). *Bythotrephes cederstroemi* appears to fit this pattern and, with their high energetic allocation to offspring, may actually be an extreme example of maternal investment by cladocerans.

The importance of early instar stages of invertebrate predators has not been emphasized in many past studies. The nauplii of predaceous copepods are not necessarily predaceous. The few studies on predaceous cladocerans, whose juvenile stages are often predaceous, primarily examined adult organisms (Butorina 1970; Hillbricht-Ilkowska and Karabin 1970; Karabin 1974; Havel 1985; Vanderploeg et al. 1993). *Bythotrephes cederstroemi* one-barb and two-barb instar individuals have higher respiration rates per unit body size, making them less efficient at energy utilization. Because of their

relatively long development times and reduced respiratory efficiency, one-barb and two-barb animals contribute significantly to the predation effect of *B. cederstroemi*. Over the time from birth to primparity, approximately 44% of an individual's total consumption occurs during the one-barb and two-barb instars.

Because they may be as much as an order of magnitude smaller than the adults, the one-barb and two-barb instar animals probably are unable to handle large prey. This suggests that their impact is greater on smaller prey items and that ontogenetic diet shifts will be an important factor in the effect of predation by the total population. Clearly the indication from Vanderploeg et al. (1993) is that, when available, *B. cederstroemi* adults obtain over half of their carbon intake from larger prey items, such as *Daphnia retrocurva*, in the Saginaw Bay zooplankton assemblage. The smaller, juvenile *B. cederstroemi* will undoubtedly include a larger proportion of smaller prey in their diets (e.g., *Bosmina*, *Ceriodaphnia*, copepod nauplii, and possibly rotifers), as observed by Mordukhai-Boltovskaya (1958) and Vanderploeg et al. (1993) for Lake Huron adults. The effect of predation by a population of *B. cederstroemi* should be twofold. First, predation by large adults on *Daphnia* should result in a shift in *Daphnia* population structure toward larger-bodied animals, as observed by Lehman (1991) in Lake Michigan. Second, predation by the smaller juveniles should result in competition with other invertebrate predators, such as *Leptodora*, for small prey such as *Bosmina*. *Bosmina* is a favored prey of *Leptodora* (Branstrator and Lehman 1991) and potentially of juvenile *B. cederstroemi*.

Bioenergetic requirements of *B. cederstroemi* can be compared with experimentally measured consumption rates of other zooplankton. The average size of a one-barb instar in our study is 33.5 μg C (67 μg dry wt.), and the predicted consumption rate of 48 μg C·d^{-1} (96 μg dry wt.·d^{-1}) results in a daily total predation rate of 143% of body weight. The model predicts that the average two-barb (73.2 μg C; 146.4 μg dry wt.) and the average three-barb (178 μg C; 355 μg dry wt.) will eat 166 and 105%·d^{-1}, respectively. Measured consumption rates for other cladocerans are varied, but comparable. For herbivorous cladocerans, Urabe (1991) found consumption in *Bosmina* to be greater than 100%·d^{-1}, ranging up to 170%·d^{-1} at high food concentrations. *Daphnia* may have maximal ingestion rates of up to 75%·d^{-1} (Lampert 1987). The predatory cladoceran *Polyphemus*, closely related to *B. cederstroemi*, consumes 86%·d^{-1} (Monakov and Sorokin 1972). *Leptodora* consumes up to 50%·d^{-1} (Karabin 1974), but also has a very high assimilation rate (87%), which may reduce the need for high consumption rates if ingestion rates are comparable with those of *B. cederstroemi*. The predatory copepod *Mesocyclops edax* consumes on the average over 100%·d^{-1} (Brandl and Fernando 1975), and *Macrocyclops albidus* consumes 86%·d^{-1} (Monakov 1972). In contrast, the invertebrate predators *Mysis* and *Megacyclops* are reported to eat only 39 (Cooper and Goldman 1980) and 26.2%·d^{-1}, respectively (average from Krylov 1988, table 3). However, their activity level is less than that of the constantly swimming *B. cederstroemi*, and their growth rates are much lower. There is a broad range of experimentally determined consumption rates between and within species, and reports of consumption rates greater than 100%·d^{-1} are common.

Predictions of prey consumption requirements, estimated from the bioenergetic needs of *B. cederstroemi* (Table 2), also can be compared with the measured clearance rates from Vanderploeg et al. (1993). The clearance rates, when converted to prey numbers, indicate that third instar *B. cederstroemi* are eating 16 *D. retrocurva*·d^{-1}. This value is substantially less than the predicted value based on bioenergetic demands. Vanderploeg et al. (1993) reported that in their enclosures *B. cederstroemi* ate 55%·d^{-1} of their body weight. Our predicted rates are twice those observed by Vanderploeg et al. (1993) and by Monakov (1972) for *Bythotrephes longimanus*. However, predation rates reported by Mordukhai-Boltovskaya (1958) are within this range (as much as 17 *Polyphemus*·d^{-1} for recently born *Bythotrephes*, probably *B. longimanus*).

There are several possible explanations for the discrepancy between our predicted bioenergetic requirements and the experimentally determined consumption rates of Vanderploeg et al. (1993). First, *B. cederstroemi* is known to be cannibalistic (Mordukhai-Boltovskaya 1958, personal observation). Cannibalism was not accounted for in computation of *B. cederstroemi* consumption rates by Vanderploeg et al. (1993; H.A. Vanderploeg, Great Lakes Environmental Research Laboratory, NOAA, Ann Arbor, Mich., personal communication). Predation by *B. cederstroemi* on conspecifics would satisfy a large portion of its consumptive demand. If a quarter of the *B. cederstroemi* were cannibalized during the experiment, the combined effect of attributing the observed predation to a larger number of animals than survived, and not accounting for the biomass of *B. cederstroemi* consumed, would make the difference between our prediction and the measurement of Vanderploeg et al. (1993) small. Second, behavioral effects in enclosure experiments are important (O'Brien 1988). We have found that short-term predation trials (24 h or less) with *B. cederstroemi* underestimate predation rates; increased rates are observed on the second and third days of incubation (unpublished). This behavioral effect of acclimation has been observed in other invertebrate predation experiments (Brandl and Fernando 1974). Third, the size of *B. cederstroemi* reported from Lake Ontario (largest adult ≃ 180 μg dry wt., Makarewicz and Jones 1990), Lake Erie (average adult ≃ 125 μg dry wt., Berg and Garton 1988, fig. 3; average adult = 155–303 μg dry wt., Garton et al. 1990), and Lake Superior (average adult <200 μg dry wt., Garton and Berg 1990) is considerably smaller than the size of *B. cederstroemi* we observed in Lake Michigan (adult range = 200–890 μg dry wt., average adult = 355 μg dry wt., this study). Vanderploeg et al. (1993), using the length–weight regression of Berg and Garton (1988), reported an average adult dry weight of 130 μg in Lake Huron. The smaller *B. cederstroemi* reported from Lakes Erie, Ontario, Superior, and possibly Huron would be expected to eat fewer prey. Finally, our model assumes *ad lib* food whereas food levels in predation experiments are constantly decreasing and may often be below saturation. Therefore, our model depicts an ideal predation environment with no food limitation, and it is not surprising that our

predictions would be higher than the consumption rates observed in some predation experiments.

We believe that the size difference between *B. cederstroemi* from Lake Michigan and those from Lakes Ontario and Erie has in part lead to some of the controversy surrounding the impact of *B. cederstroemi* in the Laurentian Great Lakes (Sprules et al. 1990; Lehman 1991). From our study, it appears that a middle ground exists. Lake Michigan *B. cederstroemi*, because of their larger size, should eat a significantly larger number of prey. These larger *B. cederstroemi* may also consume more large *Daphnia* prey than their smaller counterparts in Lakes Erie and Huron. When large *B. cederstroemi* are free from heavy fish predation and survive in large numbers, their predation may alter zooplankton community structure. Such a change in zooplankton community structure was observed in offshore areas of Lake Michigan where *B. cederstroemi* was reported in relatively large numbers (Lehman 1991). Little impact is observed in the inshore regions of Lake Michigan (Baker et al. 1992), where fish predation is more intense (Evans and Jude 1986). The smaller Lake Erie *B. cederstroemi* should eat fewer prey. Furthermore, *B. cederstroemi* abundances have not been reported to be as high in Lake Erie as in offshore Lake Michigan (Jin and Sprules 1990). *Bythotrephes cederstroemi* also may be sensitive to the higher water temperatures of western Lake Erie (Garton et al. 1990). Size, abundance, and possibly temperature differences should cause *B. cederstroemi* predation to have less impact on the Lake Erie and Lake Huron zooplankton communities than on those of Lake Michigan.

Population-level bioenergetic demands for Lake Michigan can be predicted using our results and demographic information. The sum of consumption by all instars (number of a given instar times the average daily consumption per instar) predicts the population energy demands. We know of no published demographic data for Lake Michigan *B. cederstroemi*; however, if we assume a similar population structure as was found in Lake Erie (0.22 (one-barb), 0.46 (two-barb), 0.32 (three-barb); average from Berg and Garton 1988, table 2) and use population abundance data for Lake Michigan in 1988 (Lehman 1991, fig. 1), a daily consumption rate per square metre by the *B. cederstroemi* population can be determined. Between mid-July and late August 1988, *B. cederstroemi* daily consumptive demands ranged from 16 to 86 mg $C \cdot m^{-2}$. Juvenile stages were responsible for 52.8% of the daily energy needs of the population.

Our population consumption estimate differs on average by 11% from that of Lehman and Caceres (1993, fig. 3). Their estimates are based on a simple linear extrapolation between birth weight and primaparity weight, an assumption of a constant respiration value per unit mass, and an assumed assimilation value of 80% (20% higher than our measured rate). The assumption of no allometric function for respiration results in biased estimates of respiratory demand. For example, using their own estimated respiration rates for one-barb and three-barb animals, the nonallometric approximation of Lehman and Caceres (1993) results in a 14% underestimate of one-barb respiratory demand and a 15% overestimate of three-barb respiratory demand. The use of our "average" respiration value in their calculations results in a 25% underestimate of respiratory requirements for one-barbs and a 50% overestimate of respiratory requirements for three-barbs, compared with the use of instar-specific respiratory values. Clearly, the use of a nonallometric respiration function biases the respiratory demand estimates of both juveniles and adults. In addition, the estimate of Lehman and Caceres (1993) does not take into account *B. cederstroemi* age-specific consumption rates, but uses an average daily consumption rate for all instars to calculate total population consumption. This is important, as age structure changes during the year (Berg and Garton 1988). Even correcting for differences in ingestion and assimilation parameter values, the estimate of population consumption by Lehman and Caceres (1993) for a population of *B. cederstroemi* composed primarily of one-barb animals would be 54% higher than that of our model which incorporates age structure. Similarly, if a population consisted of only three-barb individuals, their estimate would be 21% lower than that of our bioenergetic model. Finally, predicted consumption rates for each instar, rather than total population consumption estimates, are necessary to assess which prey species are likely to be affected by *B. cederstroemi* predation. The greater than fivefold variation between the average weights of one-barbs and three-barbs suggests that juveniles and adults are likely to consume different prey items. Our bioenergetic model, although requiring dry weight measurements to estimate population growth curves, can be used for populations of any age structure and provides instar-specific predation estimates.

The predicted bioenergetic requirement of the *B. cederstroemi* population is approximately equal to the average daily replacement value for herbivorous cladocerans in Lake Michigan (Lehman and Caceres 1993, fig. 3), indicating that *B. cederstroemi* populations have the potential to impose significant mortality on cladoceran populations. If *B. cederstroemi* bioenergetic demands were satisfied solely by consumption of *Daphnia galeata mendotae*, this predation would reduce the *D. galeata mendotae* population of Lake Michigan an average of 17.1% by weight per day (range 0.8%, late August 1988, to 46.5%, late July 1988; *D. galeata mendotae* abundances from Lehman 1991, fig. 1). *Bythotrephes cederstroemi* populations may have a significant seasonal impact on prey populations, at least in Lake Michigan. With growth curves for smaller *B. cederstroemi* from Lakes Erie and Huron, a similar analysis may resolve questions concerning the apparently lesser effect of *B. cederstroemi* in these lakes.

Invertebrate predation can be an important structuring process in zooplankton communities. As was demonstrated with this modeling effort, it is important to examine the entire life history of a predatory organism. Consumption by juvenile instars is a significant portion of the bioenergetic budget for *B. cederstroemi*. The large body-size difference among populations from different lakes may result in predation effects that are not directly comparable. The effect of future invasions of *B. cederstroemi* (Yan et al. 1992) on zooplankton community structure may depend on the average body size of the invading population. The consumptive demands of such an invading population would be predicted using this bioenergetic approach.

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Invasion of the exotic cladoceran *Daphnia lumholtzi* into North American reservoirs

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Abstract: *Daphnia lumholtzi*, a large cladoceran native to Africa, Asia, and Australia, has recently invaded reservoirs of the southern United States. We examined its distribution, history of invasion, and population dynamics in Missouri reservoirs. Surveys detected the species in 7 of 112 reservoirs in 1992 and 11 of 119 reservoirs in 1993. Analysis of quantitative zooplankton samples from two reservoirs over a 7-year period indicated that *D. lumholtzi* first reached detectable densities in 1990 and persisted during 1991–1993. Population maxima typically occurred in late summer, with the species absent from the plankton during winter and spring. Based on its current distributional patterns, *D. lumholtzi* appears to be capable of colonizing most reservoirs in the southern regions of North America.

Résumé : *Daphnia lumholtzi*, cladocère de grande taille indigène d'Afrique, d'Asie et d'Australie, a depuis peu envahi des plans d'eau du sud des États-Unis. Nous avons examiné sa répartition, l'histoire de l'invasion et la dynamique des populations dans les réservoirs du Missouri. Les relevés ont permis de repérer cette espèce dans 7 des 112 réservoirs en 1992 et dans 11 des 119 réservoirs en 1993. L'analyse des échantillons quantitatifs de zooplancton prélevés dans deux réservoirs sur une période de sept ans a révélé que *D. lumholtzi* a atteint des densités repérables en 1990, et a persisté en 1991–1993. Les maximums de population apparaissaient de façon caractéristique à la fin de l'été, l'espèce étant absente du plancton pendant l'hiver et le printemps. D'après son profil actuel de répartition, *D. lumholtzi* semble en mesure de coloniser la plupart des plans d'eau des régions méridionales de l'Amérique du Nord.

[Traduit par la Rédaction]

Introduction

Freshwater habitats have been invaded by a wide range of exotic species. For instance, at least 139 exotic species have invaded the Laurentian Great Lakes, and the rate of invasions has increased in recent years (Raloff 1992; Mills et al. 1993). Although some species have invaded North America by natural means, most have been purposeful or accidental human introductions (Mooney and Drake 1986). Some exotics have caused enormous problems in freshwater environments. The Nile perch, intentionally introduced to Lake Victoria, East Africa, has severely disrupted the food web and is driving native cichlids to extinction (Baskin 1992). Exotic plants, such as water hyacinths and *Hydrilla*, have clogged waterways and crowded out native plants in the United States (Culotta 1991). Zebra mussels, introduced only a decade ago to the Laurentian Great Lakes through ballast water of large freighters (Carlton 1993; Mills et al. 1993), have exploded in numbers, covering bottom substrates and clogging water intakes for industry and boats (Nalepa and Schloesser 1993).

Exotic species provide opportunities to examine the ecology and evolution of new populations (Mooney and Drake 1986). Of particular interest are characteristics of species which make them effective invaders (Ehrlich 1986), characteristics of habitats which make them susceptible to invasion ("invasibility", Ewel 1986), and influence of invaders on native food webs (Baskin 1992). Species discovered in the early stage of invasion provide unique opportunities to document these processes and their consequences.

One such species is the cladoceran *Daphnia lumholtzi* Sars, recently discovered in reservoirs of the southern United States (Sorensen and Sterner 1992; Havel and Hebert 1993). *Daphnia lumholtzi* is native to Africa, the Mideast, the Indian subcontinent, and Australia (Green 1971; Gophen 1979; Swar and Fernando 1979; Benzie 1988), where it lives in a variety of habitats ranging from deep tectonic lakes to turbid temporary ponds. *Daphnia lumholtzi* has several features which distinguish it from other *Daphnia*: a large pointed helmet and tailspine, distinctive lateral fornices, and prominent spines along the

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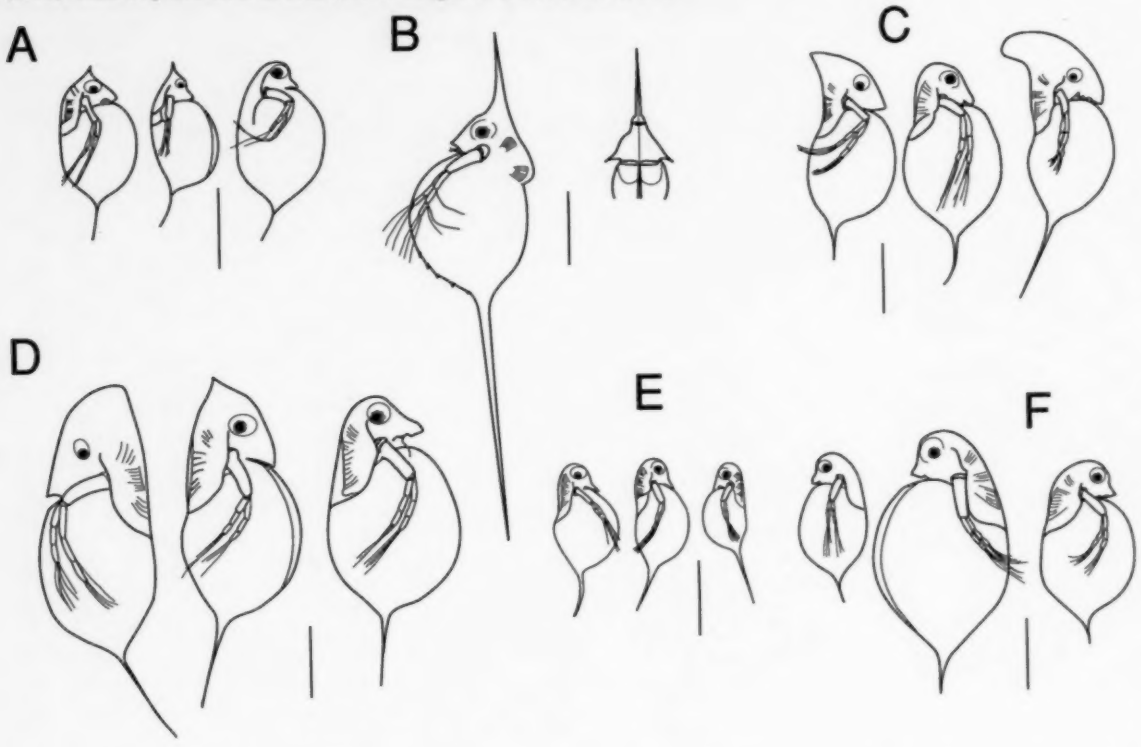
Fig. 1. Morphology of *D. lumholtzi* and other *Daphnia* common in Missouri reservoirs. A, *D. ambigua*; B, *D. lumholtzi*; C, *D. retrocurva*; D, *D. galeata mendotae*; E, *D. laevis*; F, *D. parvula*. *Daphnia lumholtzi* modified from Havel and Hebert (1993); other *Daphnia* from Brooks (1957). Length of scale bar = 0.5 mm.



ventral carapace margin (Sars 1885; Havel and Hebert 1993) (Fig. 1).

The invasion of *D. lumholtzi* is peculiar for several reasons. First, biogeographic and genetic data suggest that intercontinental dispersal of most Cladocera is ordinarily rare. Although the resistant resting eggs and parthenogenetic mode of reproduction provide a good mechanism for dispersal (Dodson and Frey 1991), most species are restricted to single continents (Frey 1982; P.D.N. Hebert, University of Guelph, Guelph, Ont., personal communication). Second, the dispersal vector is unknown. In contrast with other invertebrates which have invaded the Great Lakes via ballast of large ships (Roberts 1990; Raloff 1992; Carlton 1993), restriction of *D. lumholtzi* to inland reservoirs suggests that *D. lumholtzi* used another vector, such as introduced fishes or the aquarium trade (Havel and Hebert 1993). Finally, the species appears to have invaded numerous sites within a short time period. Recent surveys in several regions indicated that *D. lumholtzi* achieved detectable densities in early 1991 and has since invaded reservoirs in Texas, Missouri, Tennessee, and Florida (Sorensen and Sterner 1992; Havel and Hebert 1993). The recency of this invasion, together with archived zooplankton samples, provided the opportunity for a detailed study of this exotic species.

The objectives of the current study were to determine the regional distribution and population dynamics of *D. lumholtzi*, through detailed collections from Missouri reservoirs. Besides providing an early history of this invading zooplankter, these data provide a foundation for later study of range expansion and predictions of effects on native reservoir communities.

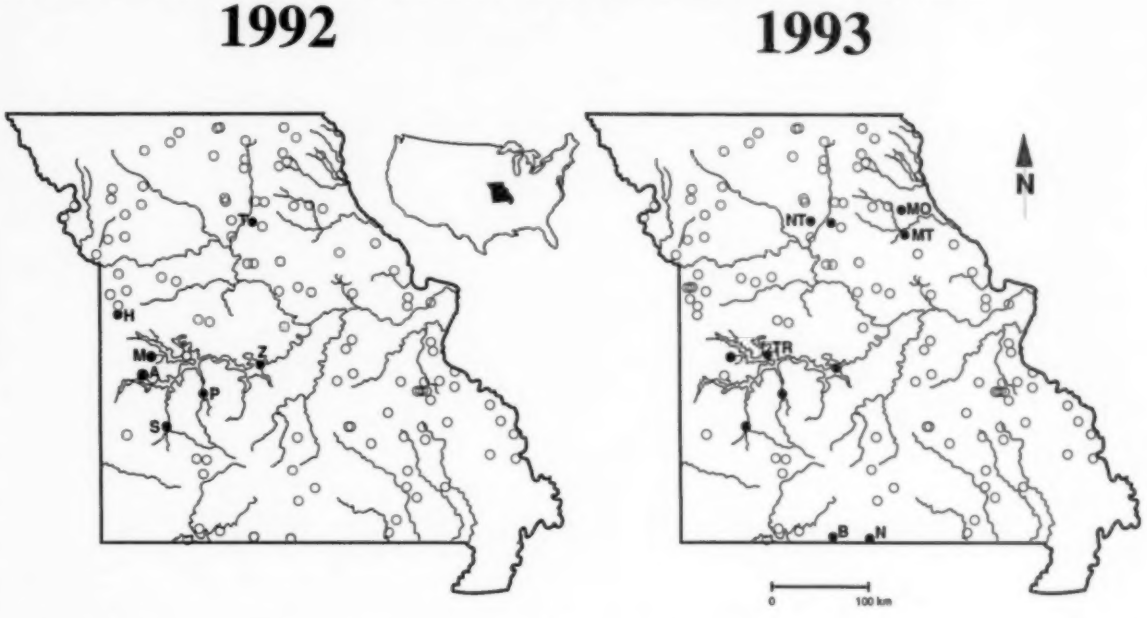
Materials and methods

Distribution in Missouri reservoirs

The distributional study involved collections from Missouri reservoirs (Fig. 2), ranging from mainstem impoundments to small tributary storage reservoirs used for water supply and recreation. The reservoirs are located in all physiographic regions of the state and exhibit a large variation in limnological characteristics (Table 1) (Jones and Knowlton 1993).

One hundred and twelve reservoirs were visited once during June 1992 and 119 were visited three times during May–August 1993. Each sample was collected near the dam, during daylight hours, by two vertical tows with a 25-cm-diameter zooplankton net (mesh = 200 μm). The two samples were pooled and preserved with buffered formalin. In order to avoid transmitting *D. lumholtzi* between reservoirs, special care was taken to thoroughly rinse collecting nets at each site. The entire sample from each site was later screened at 30× for *D. lumholtzi*. Because the sampling was not quantitative, no attempt was made to

Fig. 2. Results of Missouri reservoirs surveys for *D. lumholtzi* in 1992 and 1993. Solid circles, present; open circles, absent. Lake codes: A, Atkinson; B, Bull Shoals; H, Harrisonville; M, Montrose; MO, Monroe; MT, Mark Twain; N, Norfolk; NT, Nehai Tonkayea; P, Pomme de Terre; S, Stockton; T, Thomas Hill; TR, Truman; Z, Lake of the Ozarks.



compare densities among reservoirs. Other common cladoceran species were identified in a subset of 1992 samples, using Brooks (1957) for *Daphnia* and Edmondson (1959) for other Cladocera.

Study sites

Detailed studies of *D. lumholtzi* population dynamics were carried out in two reservoirs: Stockton and Pomme de Terre (Figs. 2 and 3; Table 2). Like most reservoirs in the Ozark Highlands, these waterbodies exhibit a warm-monomictic circulation pattern and a decrease in nutrients and increase in water clarity along an up-lake to down-lake transect (toward the dam) (Youngsteadt and Gumucio 1991). Although the two reservoirs are similar in conservative ions (alkalinity 115 $mg \cdot L^{-1}$ as $CaCO_3$, conductivity 260 $\mu S \cdot cm^{-1}$), Pomme de Terre has a shorter hydraulic retention time, higher watershed to volume ratio (5.3 vs. 2.7), and higher fertility than Stockton Lake (Table 2).

Population dynamics

Population dynamics of *D. lumholtzi* were studied through a series of quantitative zooplankton samples collected in each of seven years. On each date, four zones were sampled from Stockton Lake (Nos. 1–4, Fig. 3) and three from Pomme de Terre (Fig. 3). Within each zone, zooplankton were collected from two to four locations randomly chosen from a grid. Depth was ≥18 m in most of zone 1 for both lakes and ≤6 m for zones 2–4. During 1987–1991, zooplankton were collected weekly from May until the middle of June and then biweekly until October. During 1992–1993, samples were collected monthly from April to October. Because these zooplankton collections were made as part of an investigation of the interactions between zooplankton, gizzard shad, and sport fish (P. Michaletz, Missouri Department of Conservation, Columbia, Mo., personal communication), data on fish diets were also available for April–October of each year.

Zooplankton were collected with an 80-μm-mesh Wisconsin net, with vertical hauls to the surface, from either the bottom or 9 m, whichever distance was smaller. Recorded volume was determined by assuming that the total column of water passed through the net. Because net hauls were not calibrated with meters and net efficiency is expected to decline with increasing seston levels, the densities reported below are likely underestimates. The entire contents of each sample were examined at 25–50× and *D. lumholtzi* counted.

An additional zone on Stockton Lake (No. 5, Fig. 3) was sampled from May 1991 through December 1993 on a monthly basis, by taking three 13-m vertical tows with a 200-μm-mesh zooplankton net (total volume sampled approximately 1900 L). Prior to October 1991, subsamples were examined; thereafter, the entire contents of each sample were screened at 25× for the presence or absence of *D. lumholtzi*.

Statistical analyses

Statistical comparisons of limnological characteristics between reservoirs were carried out with Wilkoxon two-sample tests, by the methods of Sokal and Rohlf (1981), using Minitab, PC version, release 8 (Minitab 1991).

Results

Distribution in Missouri reservoirs

Examination of 1992 reservoir samples revealed nine species of *Daphnia*, of which six were common (Fig. 1).

Table 1. Limnological characteristics of Missouri reservoirs. For each reservoir, samples were collected during summer 1992 from the surface and Secchi depth and averaged. Shown are the median (and range) among reservoir samples with (n = 13) and without (n = 101) *Daphnia lumholtzi* (*D.l.*). p-values, based on Wilkoxon two-sample tests: ns, $p > 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Details on methods are presented in Jones and Knowlton (1993).

Variable	With *D.l.*	Without *D.l.*	p-value
Area (ha)	19 274 (49–147 148)	217 (25–56 833)	***
Water temperature (°C)	25.1 (23.1–27.2)	24.1 (11.6–28.1)	**
Secchi depth (m)	1.46 (0.3–2.97)	1.2 (0.2–5.3)	ns
SiO_2 ($mg \cdot L^{-1}$)	7.6 (6.5–8.9)	7.4 (4.9–11.1)	ns
Total nitrogen ($mg \cdot L^{-1}$)	0.72 (0.35–1.30)	0.73 (0.17–3.23)	ns
Dissolved nitrogen ($mg \cdot L^{-1}$)	0.60 (0.26–1.16)	0.51 (0.11–1.42)	ns
$NO_2^- + NO_3^-$ ($\mu g \cdot L^{-1}$)	48 (4–543)	5 (1–768)	***
NH_4^+ ($\mu g \cdot L^{-1}$)	31 (10–62)	31 (6–278)	ns
Total phosphorus ($\mu g \cdot L^{-1}$)	32 (13–174)	34 (6–369)	ns
Total dissolved phosphorus ($\mu g \cdot L^{-1}$)	14 (6–30)	14 (4–84)	ns
Chlorophyll *a* ($\mu g \cdot L^{-1}$)	14.9 (1.5–90.6)	12.7 (0.9–212.7)	ns
Nonvolatile suspended solids ($mg \cdot L^{-1}$)	4 (1–43)	2.7 (0.3–26.5)	ns
Volatile suspended solids ($mg \cdot L^{-1}$)	2.5 (1–11.8)	2.5 (0.5–25.4)	ns
Ca^{2+} ($mg \cdot L^{-1}$)	31 (19–62.6)	23.5 (1.7–91.7)	ns
Mg^{2+} ($mg \cdot L^{-1}$)	12.6 (4–19.3)	10.8 (1.8–82)	ns
Na^+ ($mg \cdot L^{-1}$)	4.4 (2.1–26.7)	4.6 (1–36.1)	ns
K^+ ($mg \cdot L^{-1}$)	3.9 (1.6–11.1)	3.3 (0.7–8.8)	ns
Conductivity ($\mu S \cdot cm^{-1}$)	298 (196–718)	253 (31–935)	*
Color (Pt units)	25 (15–32)	24.3 (13–56)	ns

Pelagic species richness ranged from 1 to 10 cladocerans, with up to six species of *Daphnia* coexisting in single reservoirs.

Daphnia lumholtzi was detected in 3 of the 112 reservoirs sampled in June 1992: Atkinson, Pomme de Terre, and Montrose lakes. Each of these lakes is on a separate drainage (Fig. 2). Atkinson and Montrose lakes are small (areas approximately 144 and 2675 ha, respectively), eutrophic, and turbid (Secchi depths 0.2–0.5 m). Although few individuals were detected in Atkinson and Pomme de Terre, *D. lumholtzi* was the most common zooplankter in the sample from Montrose Lake. Four additional populations were detected in samples collected in July–October 1992: Lake of the Ozarks (Niangua River arm), Stockton Lake, Thomas Hill Reservoir, and Harrisonville Lake (Fig. 2). Lake of the Ozarks is a large impoundment on the Osage River, which receives water from Stockton and Pomme de Terre lakes. Thomas Hill and Harrisonville lakes are located on separate drainages.

During May–August 1993, 119 reservoirs were each visited on three occasions. Over this period, much of the midwestern United States received exceptionally large rainfalls, resulting in widespread flooding. No *D. lumholtzi* were detected in May and June samples. During July and

Fig. 3. Sampling zones on Stockton and Pomme de Terre. Zones 1–4 were collected by the Missouri Department of Conservation and zone 5 by Springfield City Utilities.

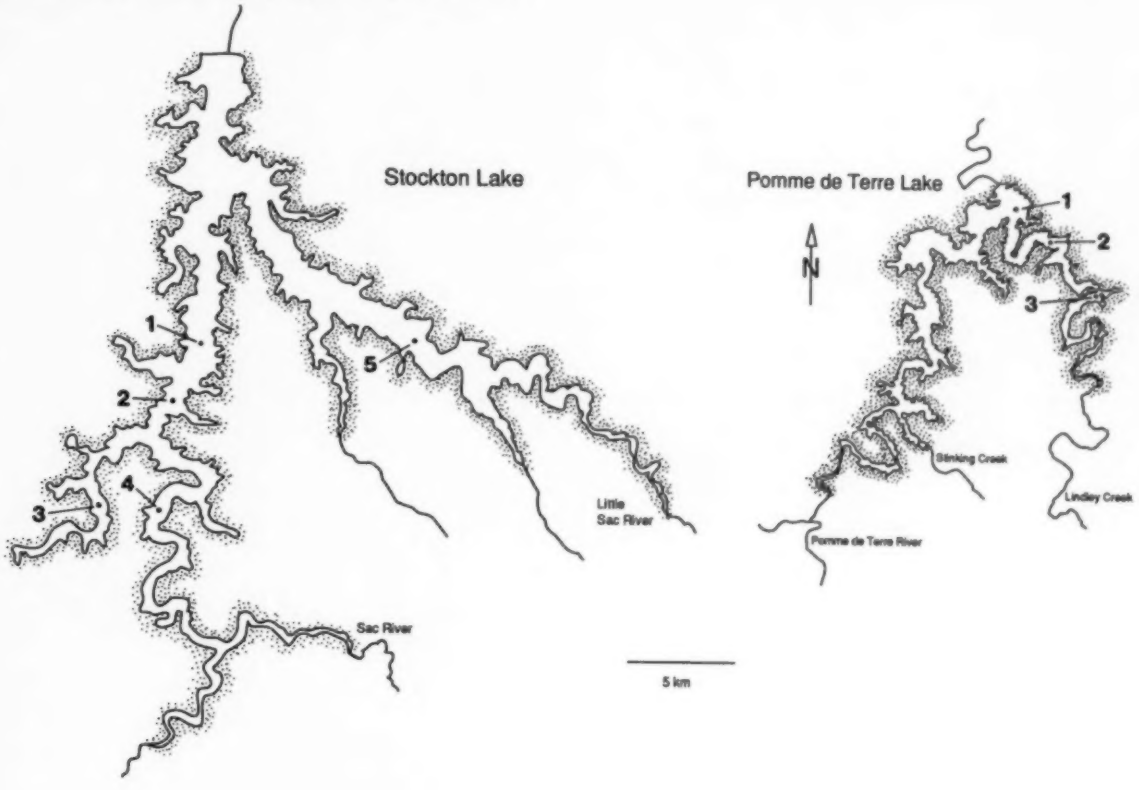


Table 2. Limnological characteristics of Stockton and Pomme de Terre lakes. For each reservoir, the water chemistry data were collected from a single site near the dam in summer 1992.

	Area (ha)	Mean depth (m)	Retention time (years)	Chlorophyll *a* ($\mu g \cdot L^{-1}$)	Total P ($\mu g \cdot L^{-1}$)	Total N ($\mu g \cdot L^{-1}$)	Secchi depth (m)
Stockton	10 031	10.9	1.20	5.1	13	400	3.0
Sac River arm	2 072	7.3	0.31				
Little Sac arm	3 423	10.8	1.12				
Pomme de Terre	3 194	9.3	0.60	13.9	20	520	1.7
Lindley Creek arm	672	8.8	0.46				
Pomme de Terre arm	950	12.6	0.42				

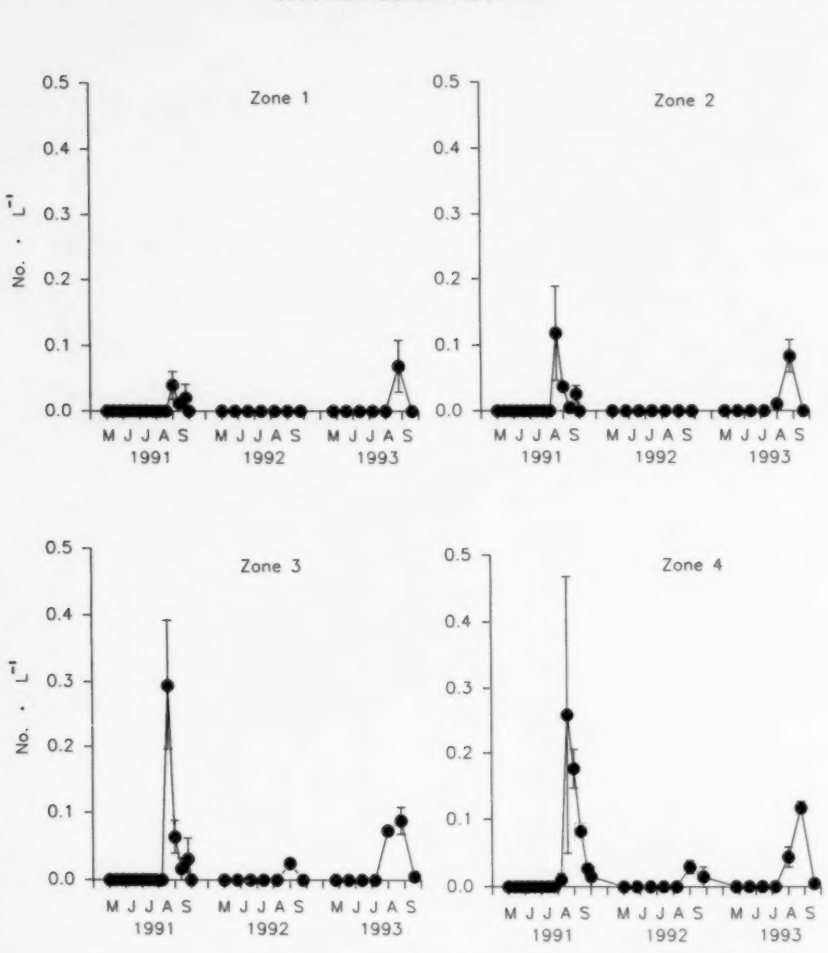
August, populations were detected in 11 reservoirs. Six of these represented populations not detected in 1992, although one reservoir (Mark Twain) had not been sampled in 1992. Five populations were detected in both 1992 and 1993 (Fig. 2). Despite the extensive sampling, no *D. lumholtzi* were detected in Atkinson or Harrisonville Lake, indicating that these populations were either locally extinct or extremely rare.

Based on both years, at least 13 Missouri reservoirs have been invaded so far by *D. lumholtzi*. There were no significant differences in median values for most limnological characteristics of the 13 invaded reservoirs and 101 others which have not been invaded (Table 1). However, invaded reservoirs tended to be larger in area, to have a higher water temperature, and to have higher conductivities and higher concentrations of nitrite and nitrate than noninvaded reservoirs.

Population dynamics in Stockton and Pomme de Terre

The zooplankton communities of the two lakes consisted of 29 species, including six species of *Daphnia* (Fig. 1).

Fig. 4. Population dynamics of *D. lumholtzi* in Stockton Lake (mean ± 1 SE (n = 3)).



Native *Daphnia* species typically reached their maximal abundances during spring and early summer and *Ceriodaphnia* and *Diaphanosoma* species during late summer (J. Havel and W. Mabee, personal observation).

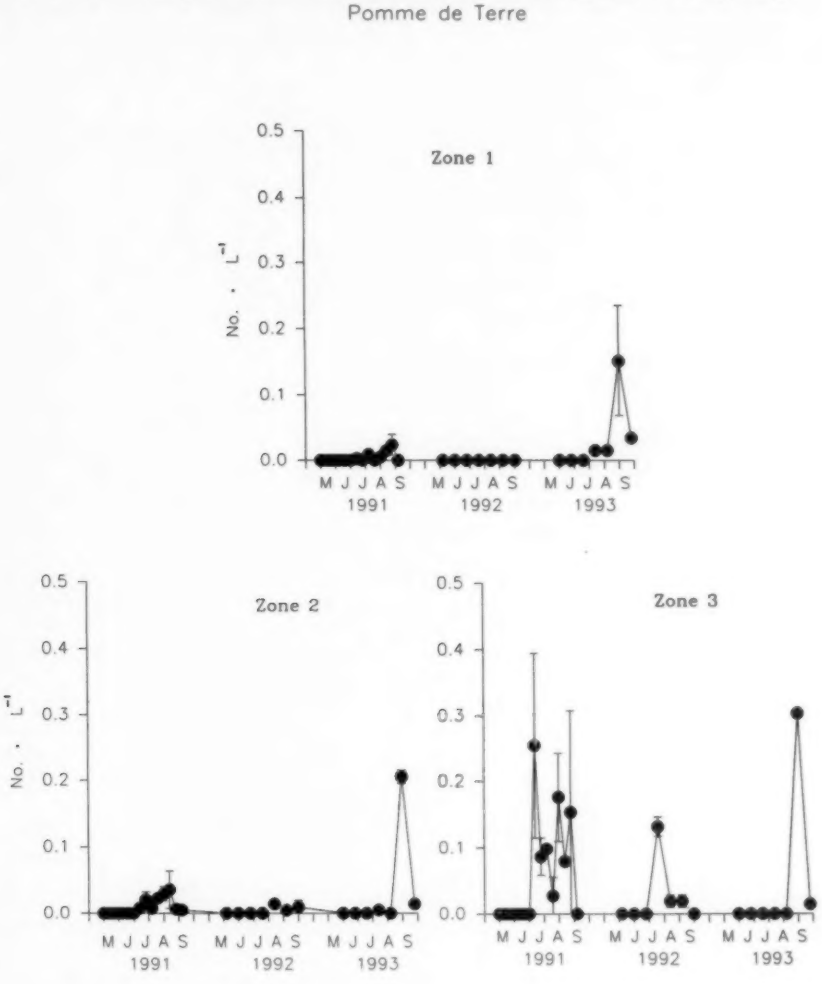
Daphnia lumholtzi was not observed in any sample in 1987, 1988, 1989, or early 1990. In Stockton Lake, this species first appeared in a stomach sample from a white crappie (*Pomoxis annularis*) on August 14, 1990, and in a zooplankton sample on October 1, 1990. In Pomme de Terre, *D. lumholtzi* first appeared in a white crappie stomach sample on October 11, 1990, and in a zooplankton sample on October 3, 1990, when it was the dominant cladoceran.

Population density estimates for Stockton Lake, based on samples collected between 1991 and 1993, indicated that *D. lumholtzi* was rare or absent during each spring, suddenly increased in late summer, and declined in autumn (Fig. 4). The pattern was evident at all four zones. Although population densities appeared to decline from 1991 to 1992, *D. lumholtzi* were still abundant in 1993 (Fig. 4). Monthly samples collected over three years at an additional up-lake site (No. 5) showed a similar pattern, indicating that this species was absent from the plankton during all months except August–October. Samples collected from Pomme de Terre also indicated a short summer population peak for *D. lumholtzi* in most years, although in one year (1991) the density remained high for most of the summer (Fig. 5). For both lakes, densities were greatest at up-lake zones (Nos. 3 and 4), and this pattern was consistent for most dates (Figs. 4 and 5).

Discussion

We have shown that the exotic *D. lumholtzi* has so far invaded approximately 11% of the reservoirs in Missouri and has persisted in two well-studied reservoirs for at least 3 years. Although the 2-year survey suggests that a range expansion is taking place, the larger number of populations detected could have been an artifact of the increased sampling effort in 1993. The observation that two populations

Fig. 5. Population dynamics of *D. lumholtzi* in Pomme de Terre (mean ± 1 SE (n = 3)).



detected in 1992 were apparently extinct in 1993 suggests that populations are unstable or extremely patchy. Their brief period of population abundance each year may make *D. lumholtzi* sensitive to environmental disturbances. For instance, Atkinson Lake, which had a small population in 1992, was inundated by (the normally downstream) Truman Lake during the severe flooding of 1993, bringing in a large population of gizzard shad (*Dorosoma cepedianum*; D. Cornelius, Missouri Department of Conservation, Sedalia, Mo.). These planktivorous fish or other flood-associated changes may have kept *D. lumholtzi* below detectable limits in 1993.

These reservoirs need monitoring to determine if established populations will persist and if the range of this species will continue to expand. We expect that reservoirs located downstream or in close proximity to established populations will have the highest probability of being colonized. Further, if human activity is the primary vector for moving propagules among sites, as suggested by Havel and Hebert (1993), then we would expect that reservoirs which receive frequent boat traffic from sites with established populations should also have a high probability of colonization. This "bass-boat hypothesis" could be further tested by sampling live wells of fishermen moving among sites, just as ballast tanks have been sampled from large ships (Carlton and Geller 1993; Locke et al. 1993). Because our study revealed that the population size of *D. lumholtzi* is largest in late summer, such distributional studies should focus on samples collected at this time of year.

Studies of range expansion of new exotics also provide a model system for estimating rates at which zooplankton colonize new habitats. Because of the presence of a long-lasting egg bank in the sediments (Dodson and Frey 1991), it is usually difficult to separate colonization of new habitats from the hatching of eggs from earlier populations. In contrast, all new occurrences of exotic species, because they did not previously occur in the region and did not have an egg bank, would represent true colonization events.

Exotics thus serve as a good marker for determining zooplankton dispersal. Such studies may yield insight into questions about how zooplankton communities are reestablished in previously degraded systems (Keller and Yan 1991).

Successfully invading species often share similar life-history traits. They generally have broad environmental tolerances, short generation times, and are habitat and trophic generalists (Ehrlich 1986). *Daphnia* and other cladocerans have many of these properties, suggesting that this group should be effective at dispersal and colonization. Specifically, they are abundant in their native range, have short generation times, and a single individual (or egg) is able to colonize alone (Dodson and Frey 1991). The observation that most cladoceran species are limited to single continents (Frey 1982) suggests either that long-distance dispersal is usually not very effective or that colonization of filled niches is difficult. *Daphnia lumholtzi* is unusual in having a native distribution over three continents, residing in a wide assortment of habitats, from clear deep lakes and temporary ponds to turbid impoundments and floodplains (Green 1967; Gophen 1979; Geddes 1984). Such a broad habitat range may have contributed to its success in rapidly establishing multiple populations in North America.

Certain communities may be especially prone to invasion by exotic species: disturbed or cultivated habitats have long been recognized to be particularly vulnerable to invasion (Elton 1958). Similarly, the fluctuating water levels, habitat alteration, turbidity, and intentional food web manipulations make reservoirs highly disturbed systems (Thornton et al. 1990) and, we would expect, particularly susceptible to establishment of invading species. It should be no surprise that *D. lumholtzi*, without the ballast-water vector used by Great Lakes exotics (Locke et al. 1993; Mills et al. 1993), should invade reservoirs rather than natural lakes. However, such a pattern could be simply an artifact, since there are few natural lakes in the areas studied.

The current study allowed testing for an association between the occurrence of successful invasion by *D. lumholtzi* and limnological characteristics. There appears to be a broad overlap of most limnological characteristics in the 13 Missouri reservoirs invaded and 101 reference reservoirs not invaded (Table 1). For instance, with the exception of nitrite/nitrate, all measures of fertility were similar between the two groups. In contrast, the area of invaded reservoirs tended to be larger than that of those not invaded. Such an observation is consistent with a variety of expectations from species–area curves (Dodson 1992). For instance, dispersal vectors such as waterfowl or boats should have a higher probability of encountering larger lakes than smaller lakes. Similarly, more open niches might be available in larger lakes than smaller lakes, providing for a lower probability of extinction.

Reservoirs invaded by *D. lumholtzi* were, on average, 1° warmer than those not invaded (Table 1). These survey data are consistent with the population dynamics in Stockton and Pomme de Terre, as populations developed only during the warm summer months and highest densities were found at the shallower (and warmer) up-lake stations. These data suggest that *D. lumholtzi* will be restricted to lakes that develop warm epilimnetic temperatures during the summer. A large number of temperate and tropical lakes in North America should thus be susceptible to invasion by this exotic.

Community composition should also influence invasibility (Ewel 1986). In the current study, populations of *D. lumholtzi* were most abundant during periods when most native *Daphnia* were rare. Summer maxima for *D. lumholtzi* were considerably lower than those which native *Daphnia* achieved earlier in the year. For instance, whereas we observed a maximum of <0.5 *D. lumholtzi*·L^{-1} (Figs. 4 and 5), native *Daphnia* during the spring bloom in Stockton Lake (primarily *D. galeata*; J. Havel, personal observation) can often exceed 20 individuals·L^{-1} (N. Youngstead, City Utilities of Springfield, personal communication). The lower density of *D. lumholtzi* could be due to lower quality food typical of late summer (Wetzel 1983) or to a variety of competitive and predatory interactions with zooplankton and planktivorous fish. It is intriguing to note that coincident with the lower abundances of *D. lumholtzi* in 1992 (Figs. 4 and 5) were large midsummer populations of native *Daphnia* (N. Youngsteadt, personal communication). Perhaps *D. lumholtzi* is restricted to living during a period in which its competitively superior congeners are normally absent. Clearly, more work is necessary to elucidate the relationship between community composition and population cycles in *D. lumholtzi*.

Some exotics have caused widespread disruption of native freshwater food webs. The best known cases involve the importation of top predators, such as Nile perch in Lake Victoria (Baskin 1992) and peacock bass in Lake Gatun (Zaret and Paine 1973). Few data exist on exotic zooplankton and their impacts on aquatic food webs. *Bythotrephes cederstroemi* is the best-known example of a cladoceran invading North American lakes. This predatory species appears to have invaded the Great Lakes in the late 1970's or early 1980's, and, like zebra mussels, has been linked to the ballast water of ships (Lehman 1987). Although widespread disruption of pelagic food webs has not yet been detected (Sprules et al. 1990), *B. cederstroemi* abundance has been linked to a depression of another predatory cladoceran (*Leptodora kindti*), as well as two of three common *Daphnia* species living in Lake Michigan (Lehman and Cáceres 1993). A possible collapse of *Daphnia* populations is potentially important because of its role as a "keystone herbivore" in freshwater systems (Stockner and Porter 1988).

Daphnia lumholtzi also has the potential for disrupting community structure. This herbivorous species has both an enlarged pointed helmet and extremely long tailspine (Fig. 1). Similar structures have been experimentally shown to reduce predation by both invertebrates (O'Brien et al. 1979) and small planktivorous fish (Jacobs 1967; Barnhisel 1991). Green (1967) found both helmeted and nonhelmeted ("monacha") morphs of *D. lumholtzi* living in Lake Albert, Uganda. He noted that the monacha morph was rarely found in locations with high densities of planktivorous fish whereas the helmeted morph was commonly found in these locations. Stomach contents from *Alestes baremose* from Buhuka Lagoon, where the two morphs cooccurred, contained a large volume of the monacha morph, but none

of the helmeted morph. Based on these data, Green (1967) concluded that the helmet and/or spines reduced predation on this morph. If North American *D. lumholtzi* should outcompete native zooplankton and become dominant during periods when larval and juvenile fishes are feeding on zooplankton, then this exotic could negatively impact the fisheries of North American reservoirs. So far, however, *D. lumholtzi* has been most abundant late in the summer, after the most active period of planktivory by larval fishes (P. Michaletz, personal communication), so this exotic has so far had no discernable impact.

In conclusion, the rapidity with which *D. lumholtzi* has invaded North America so far suggests that this species will eventually become a common member of the zooplankton in reservoirs of southern North America. Study of its progression, together with possible impacts on native food webs, should provide a fascinating look into the ecology of biological invasions.

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Lipid composition of tissue and plasma in two mediterranean fishes, the gilt-head sea bream (*Chrysophyrys auratus*) and the European seabass (*Dicentrarchus labrax*)

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Abstract: The fatty acid (FA) composition of phospholipid (PL), triacylglycerol (TAG), and total lipid (TL) fractions was determined for liver, adipose tissue, white muscle, and plasma of European seabass (*Dicentrarchus labrax*) and gilt-head sea bream (*Chrysophyrys auratus*) fed on the same diet. FA composition of plasma nonesterified fatty acids (NEFA) and TAG as well as tissue TAG and TL correlates with that of the diet. FA composition of PL in tissues and plasma was different from that of the diet and different in the two species. Contribution to tissue wet weight of the different lipid fractions showed that both species store lipid primarily outside of muscle (only 3% in both species) as TAG in adipose tissue (about 80% lipid·wet weight^{-1}). Seabass store 33% wet weight of lipid in liver, while sea bream store only 5% wet weight. The plasma concentration of NEFA and TAG is 2223 and 12 260 nmol·mL^{-1} for seabass and 2790 and 9670 nmol·mL^{-1} for sea bream. The data show that these two fishes with similar lifestyle store lipid in the same fashion and that dietary FA composition affects the FA composition of TL and TAG fractions but not PL in tissue and plasma.

Résumé : Nous avons mesuré la composition en acides gras (FA) des phospholipides (PL), des triacylglycérides (TAG) et des lipides totaux (TL) dans le foie, le tissu adipeux, le muscle blanc et le plasma chez le bar commun (*Dicentrarchus labrax*) et la dorade royale (*Chrysophyrys auratus*), ces deux poissons recevant la même alimentation. La composition en AG des acides gras libres (NEFA) et de TAG du plasma ainsi que de TAG et de TL des tissus correspond à la proportion observée dans le régime alimentaire. Par contre, la composition en FA de PL dans les tissus et le plasma était différente de celle du régime alimentaire, et différait chez les deux espèces. La contribution des différentes fractions lipidiques au poids humide des tissus révèle que les espèces stockent les lipides essentiellement à l'extérieur du muscle (3 % seulement chez les deux espèces) sous forme de TAG dans le tissu adipeux (environ 80 % des lipides, en poids humide). Le bar stocke 33 % en poids humide des lipides dans le foie, contre 5 % seulement chez la dorade. La concentration plasmique de NEFA et de TAG est de 2 223 and 12 260 nmol·mL^{-1} pour le bar et 2 790 and 9 670 nmol·mL^{-1} pour la dorade. Les données montrent que ces deux poissons au mode de vie similaire stockent les lipides de la même façon, et que la composition du régime alimentaire en FA agit sur la composition en FA de TL et de TAG mais non sur celle de PL dans les tissus et le plasma.

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Introduction

Lipids serve many roles in vertebrates. Among the most prominent are (1) an energy source and (2) the main component of cellular membranes. They are stored primarily as triacylglycerols (TAG), whole-body stores of which represent the largest energy source for fishes (Moyes and West 1994). Phospholipids (PL), the major component of cell membranes, help maintain membrane fluidity and function despite changes in water salinity and/or temperature (Bell et al. 1986). The major lipid storage sites in fish are liver, adipose tissue, and skeletal muscle. The principal

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lipid storage organ varies greatly among fish species. Some species store most of their TAG in muscle, while others use either the liver or adipose tissue as their principal site for lipid (Sheridan 1988). It has been suggested that differences in storage site may be attributed to life-style. Sedentary fish tend to store lipid in liver and/or adipose tissue, relying heavily on the circulation for transport, while more active fish use muscle for fat storage where it is more readily available to power locomotion (Larsson and Fange 1977; Weber and Zwingelstein 1994).

Plasma is used as the means of transport from either the gut or storage organ to catabolic or storage tissues and in so doing can also serve as a storage site itself. The major circulatory lipid class in fish are nonesterified fatty acids (NEFA), which are used for rapid delivery, whereas TAG and PL are used for slow delivery. Of the circulating NEFA, 20–30% are unsaturated, having carbon chain lengths of 20–22; their major function is to serve as precursors for PL and eicosanoid compounds. Monoenes and saturated NEFA of shorter chain lengths (18 C and less) are thought to be important for energy production, and those present in the plasma in highest concentrations should be those preferred by catabolic tissues. There is some evidence of preferential oxidation of these fatty acids (FA) at the level of red muscle mitochondria (Kiessling and Kiessling 1993). TAG, PL, and cholesterol must also play an important role in energy metabolism because they can contain 10–40 times the energy stored in circulating NEFA (Weber and Zwingelstein 1994).

Dietary lipids, specifically in respect to their FA composition, are thought to affect the NEFA composition of fish plasma (Henderson and Tocher 1987) and may contribute directly to blood NEFA, although this is not well established (Greene and Selivonchick 1987). This suggests that, like nonruminant mammals, fish digest lipids with very little preabsorptive modification of individual FA and that, like mammals, their adipose tissue (TAG) FA composition (Argenzio 1984) and consequentially their plasma NEFA composition (Jéquier 1992) should reflect their dietary FA composition.

The purpose of this study was to see if two fish species with similar life-styles and also fed the same diet had (1) similar lipid storage sites and (2) similar FA compositions in their different lipid classes.

Materials and methods

Experimental animals

Gilt-head sea bream (*Chrysophyrys auratus*) and European seabass (*Dicentrarchus labrax*) were originally obtained from a sea farm (Centre de production aquacole IFREMER, Palavas les Flots) and were kept in indoor tanks with continuously flowing seawater at 22°C. Males from both species were used; they were at least 2 years old and weighed 200–300 g. Experiments were carried out in July and August 1992.

Diet

Both species were fed commercial sea fish feed (Aqualim, Nersac, France; 49% crude protein, 10% crude fat, 3% crude cellulose, 13% crude ash). Feeding continued up until the time of the experiments. The total lipid content of the feed was found to be 10.6%. Lipids were extracted from feed for analysis using chloroform–methanol (Folch et al. 1957) and total FA were liberated by saponification for 3 hours at 80°C. Nonsaponifiable contaminants were removed with hexane extraction and the saponified FA were acidified with 6 N HCl to convert them back to free fatty acids (FFA). FFA were extracted with hexane and the solvent was evaporated under nitrogen. Methylation of FFA for analysis by gas–liquid chromatography (GLC) was performed with acetyl chloride (0.5 M) in anhydrous methanol and benzene.

Plasma

Extraction of lipids and methylation of FFA

Blood samples were obtained either via the caudal vein or heart puncture with a 19-gauge needle. Blood was centrifuged and plasma samples were frozen immediately. Lipids were extracted and plasma NEFA were methylated as previously described (Tserng et al. 1981) with modification to improve extraction (see below).

In a Teflon screw-capped glass culture tube, 200 μL of plasma was mixed with 100 μL of internal standard heptadecanoic acid (12 μg·100 μL methanol^{-1}). 2,2-Dimethoxypropane (DMP) was added (2 mL) as a water scavenger, deproteinizing agent, and methylating agent. (Unlike other techniques (see Hallaq et al. 1993), DMP converts FFA to their methyl esters but is not reactive enough at low temperatures to transmethylate esterified FA from TAG or PL, which remain unmodified as shown by thin-layer chromatography (TLC) (unpublished data).) Hydrochloric acid was then added (40 μL) and the resulting reaction was allowed to proceed at room temperature for a minimum of 40 minutes to allow all FFA to be methylated. (This time was determined to be sufficient for complete methylation of NEFA by using standard mixtures of NEFA and comparing GLC analysis with those of classic methods using separation by TLC and methylation with methanol–HCl (unpublished data).) The reaction was then stopped by the addition of 20 μL of pyridine and the mixture was evaporated to 100–200 μL using a heated water bath and a steady of nitrogen. Distilled water (1 mL) was added and all lipids were extracted twice with 2.5 mL of 2,2,4-trimethylpentane (isooctane). The isooctane extracts were evaporated to dryness and then redissolved in 50 μL of isooctane, 2.5 μL of which was used for gas chromatography of methyl esters.

FA composition of plasma PL and triglycerides

TL were extracted from plasma and methylated as described previously (Anderton et al. 1982). Triglycerides and PL were also separated on silica TLC plates (Riedel-de Haen silica gel 60 special) using a solvent mixture of heptane – isopropyl ether – acetic acid in the proportions 60:30:3. Lipids from approximately 100–200 μL of plasma were deposited on the TLC plate and after solvent migration the plates were dried and sections containing PL and triglycerides that were revealed under UV using a primuline reagent (Skipski 1973) were scraped off and placed in separate tubes. To each tube, 0.5 mL of a benzene–HCl mixture

(60:30) was added followed by 2.5 mL of 0.5 M HCl in methanol. The tubes were incubated at 80°C for 1.5 h. Methyl esters of FA were extracted twice with 3 mL of hexane and a final time with 1.5 mL. The combined hexane extracts were evaporated to dryness and methyl esters were redissolved in 50 μL of hexane for analysis by GLC.

Total triglyceride

The concentration of plasma total triglyceride was determined spectrophotometrically (SP 6550 UV/VIS PYE UNICAM) using an enzymatic procedure (Sigma Chemical, St. Louis, Mo.). Plasma (10 μL) was placed in a cuvet with 0.8 mL of triglyceride working reagent (triglyceride (GPO trinder) reagent A and triglyceride reagent B in a proportion of 4:1). Triglyceride standards (Sigma Chemical, St. Louis, Mo.) at concentrations of 50, 75, 100, 115, and 130 $\mu g \cdot mL^{-1}$ were used to generate a calibration curve. Concentrations in micrograms per millilitre were converted to moles using an average molecular weight for TAG calculated from the FFA composition of plasma TAG determined by GLC (930 for seabass and 925 for sea bream).

Tissues

Extraction of lipids from liver and muscle

After fish were killed by decapitation, and samples of their anterodorsal white muscle, liver, and adipose tissue (mesenteric fat, sea bream only) taken immediately, approximately 2 g was placed in Folch solution (chloroform–methanol (2:1)) at a volume of 20 $mL \cdot g\ tissue^{-1}$ (Folch et al. 1957). Tissues were homogenized using a Polytron homogenizer (PT OD 10) that was rinsed after each sample with 10 mL of Folch 2:1. The rinse solution was added to the homogenate. Homogenates were left at room temperature for about 1 h to ensure that all lipids were extracted. Each solution was then filtered and the tubes rinsed with 10 mL of chloroform–methanol (2:1). Aqueous 0.25% KCl solution was then added to the filtrate at a volume one quarter that of the final volume of Folch solution (17.5 mL in most cases) after which the tube contents were mixed. The phases were separated by placing the tubes in a heated water bath (50°C) for several minutes; the top phase containing water, methanol, and water-soluble nonlipid contaminants was removed and discarded. The proportion of chloroform–methanol was restored to 2:1 by the addition of 23 mL (one third total volume) of methanol and a second wash was carried out by the addition of 17.5 mL of distilled water (one quarter total volume). Again the layers were separated and the top layer was discarded.

The lipid extracts were transferred to a tared 250-mL evaporating flask and the solvent was evaporated at 60°C under vacuum using a Rotovap apparatus (Buchi). A second evaporation was performed after the addition of 10 mL of 99% ethanol used to eliminate any remaining water by forming an azeotropic mixture. The flask contents were dried a final time by using a high-vacuum oil pump and allowed to equilibrate to room temperature before weighing. Lipid weight is expressed as a percentage of wet weight of the fish tissue. Lipids were redissolved in 5 mL of benzene–methanol (2:1), 50 μL of which was taken for muscle and 25 μL for liver to determine total lipid phosphorus. The remaining extracts were transferred to Teflon screw-capped glass culture tubes for storage at −30°C.

Total lipid, triglyceride, and PL FA composition

An aliquot of tissue lipid extract (approximately 1 mg of total lipids) was methylated directly with a HCl–methanol mixture (1 M) to determine total lipid FA composition. Triglycerides and PL were separated by depositing approximately 1–2 mg of total lipids on a TLC plate and using isopropyl ether as the mobile phase to separate polar lipids from neutral lipids. Free cholesterol was also separated, remaining between PL and triglycerides on the silica gel. The sections of the silica gel containing TAG and PL were removed, placed in glass culture tubes with Teflon-lined screw-caps, and methylated as described earlier. Total lipid methylated FA were redissolved in 200 μL of hexane, and TAG and PL FA methyl esters were redissolved in 100 μL for analysis by gas chromatography.

The concentration of PL in both tissue and plasma lipids was determined by the Bartlett (1959) method as modified by Portoukalian et al. (1978). The total phosphorus concentration was calculated by taking the lipid phosphorus, expressed in milligrams per 100 g of tissue, and multiplying by 27 (coefficient previously determined by assaying the fish lipid phosphorus of PL fractions separated by column liquid chromatography).

Gas chromatography

Methyl esters of FA were analyzed using a Chromopack gas chromatograph model CP 9000 equipped with a flame ionization detector (FID). Analysis was performed on an 80-m capillary column coated with a liquid phase consisting of a film of CP Sil 88 (100% cyanopropyl-polysiloxane). The column was held at 190°C for the first 5 min of each run and was then raised at $2°C \cdot min^{-1}$ for 17.5 min to 225°C. The column was held at this temperature for 5 min before increasing to the final temperature of 235°C at a rate of $10°C \cdot min^{-1}$. The oven temperature remained at 235°C until component elution was complete. Injection port and detector temperatures were 250 and 275°C, respectively. Nitrogen was used as the carrier gas. The gas chromatograph was calibrated with FA standards PUFA 1, PUFA 2, rapeseed oil, and GC 90 (Supelco).

Statistics

Means for individual NEFA were compared using a one-way ANOVA. Percentages were arcsine transformed. If test for normality failed, then a nonparametric Kruskal–Wallis one-way ANOVA on ranks was used. All values presented are means ± SEM.

Results

Dietary FA

The FA composition of the dietary lipids appears in Table 1. The feed was high in both saturated, monounsaturated, and polyunsaturated FA, especially 16:0 at 16%, 18:1(n - 9) at 15%, 20:5(n - 3) at 11%, and 22:6(n - 3) at 17% (Table 1).

Plasma NEFA, TAG, and PL

Concentrations of NEFA in plasma are similar in the two species at 2790 ± 62 and 2223 ± 32 $nmol \cdot mL^{-1}$ in seabass

Table 1. FA composition (%) of commercial feed lipids (Aqualim, Nersac, France) fed to seabass and sea bream.

FA	%
14:0	2.1 ± 0.3
16:0	15.8 ± 0.8
16:1(*n*-7)	4.5 ± 0.7
18:0	4.4 ± 0.2
18:1(*n*-9)	15.3 ± 1.6
18:2(*n*-6)	5.6 ± 0.2
18:3(*n*-3)	1.5 ± 0.1
20:1(*n*-9)	3.2 ± 0.2
20:4(*n*-6)	4.2 ± 0.1
22:1(*n*-9)	1.7 ± 0.4
20:5(*n*-3)	10.5 ± 0.4
22:4(*n*-6)	1.4 ± 0.1
22:5(*n*-3)	1.6 ± 0.3
22:6(*n*-3)	16.9 ± 1.5
Saturated	22.3
Monoene	23.0
Diene	5.6
Triene	1.5
PUFA	36.3
n-3	32.2
n-6	11.2
n-3/*n*-6	2.9

and sea bream, respectively ($p > 0.05$). Seabass, however, have much higher total TAG concentrations, 12 260 ± 90 vs. 9670 ± 580 nmol·mL^{-1} in sea bream ($p = 0.002$; Table 2).

Plasma NEFA composition as well as FA composition for TAG and PL fractions for both seabass and sea bream appear in Table 2. The two species have similar FA compositions in their plasma NEFA, TAG, and PL fractions. All three fractions in both species have high proportions of palmitate (16:0), giving high total saturated FA values (24–30%). NEFA and TAG in both species are also high in oleate (18:1(*n* - 9)), while PL is significantly lower in this FA ($p < 0.05$, ANOVA). All fractions are high in monoenes (15–45%), and polyunsaturated FA (24–58%), particularly *n* - 3 (20–55%). NEFA and PL in both species are high in docosahexaenoate (22:6(*n* - 3)), but TAG is lower in this FA ($p < 0.05$).

Tissue TL, TAG, and PL

TL, TAG, and PL FA compositions for liver, muscle, and adipose tissue, the main lipid storage organs, appear in Tables 3, 4, and 5. Liver is a major lipid storage organ in the seabass, with 34.0 ± 1.4% of its wet weight made up of lipid (31.0 ± 2.1 exists as TAG), and makes up on average 2.1 ± 1% of their body weight. In comparison, sea bream liver is only 4.8 ± 0.2% lipid per wet weight and makes up a lower percentage of their total body weight at 1.7 ± 0.2% (Table 3). All fractions were high in saturated FA, especially 16:0. Monoenes were high in the TL fraction of both species and the TAG fraction of sea bream, mainly due to high oleate values. PUFA were low in TL in seabass having much lower 22:6(*n* - 3) levels when compared with PL ($p < 0.05$) or sea bream TL values. The 22:6(*n* - 3) values were comparable in the seabass TL and sea bream TAG. PUFA values were relatively high in seabass PL, and in all three fractions (TL, PL, and TAG) in sea bream, *n* - 3 PUFA values were again high (Table 3). There were, however, differences between these species in many of the individual FA levels of PL leading to differences in dienes and trienes.

The major storage site for lipid in both these species is adipose tissue. Sea bream adipose tissue ranges from 1.5 to 2.5% of body weight, depending on the season, and is 81% lipid by wet weight (Table 3). Seabass are also high in adipose tissue, with adiposity (adipose tissue/body weight) ranging from 3.5 ± 0.8 to 6.5 ± 0.6% body weight, again depending on the season. Their adipose tissue is, on average, 81.3 ± 4.2% TL per tissue wet weight, with 81% of this existing as neutral lipid (mostly TAG, unpublished data). Adipose tissue TL in sea bream was high in palmitate (16:0) at 13%, oleate (18:1(*n* - 9)) at 27%, helping to give high monoene levels, and docosahexaenoate (22:6(*n* - 3)) at 13%, leading to high PUFA values. These are close to values for dietary NEFA (see Table 1).

Both species stored about the same amount of lipid in their muscles (3.05 ± 0.35 and 3.17 ± 0.53% lipid by wet weight in seabass and sea bream, respectively; Table 5). As seen in the liver, all lipid fractions were high in saturated FA, especially 16:0. The most abundant FA in TL fractions of both species was 18:1(*n* - 9) which produced high total monoene levels. Both species had high levels of palmitate and oleate in TAG, but sea bream had higher levels of 22:6(*n* - 3). PL had significantly lower levels of oleate in both fish ($p < 0.05$). PL was, however, much higher in 22:6(*n* - 3) ($p < 0.05$) giving them higher PUFA and *n*-3 fatty acid levels. Again there were some differences between the two species in individual FA of PL (Table 5).

Discussion

The results show that two species with similar low-activity life-styles store lipids primarily outside of locomotory muscle, in liver and/or adipose tissue. Two species fed the same commercial diet have similar FA compositions in all plasma lipid fractions, except PL, as well as TAG and TL fractions in liver and muscle. There were, however, differences in individual FA of the PL fractions between these two species and their diet.

Lipid storage

These species have about the same mass and similar life-styles. Both are benthic and live at a depth of 50–90 m near the bottom of the Mediterranean Sea (UNESCO 1986). They resemble related North American fish species *Morone saxatilis* and *Stenotomus chrysops* considered to be only moderately active owing to their gill surface area to body mass ratio (Gray 1954). As hypothesized for less active fish (Weber and Zwingelstein 1994), the fish we have studied store their lipids primarily in organs other than muscle: liver and adipose tissue for seabass and mainly adipose tissue for sea bream (Tables 3 and 4).

Table 2. Plasma FA composition (%) of NEFA, TAG, and PL in seabass and sea bream (n = 5). Values are means ± SEM. ND = not detectable.

	Seabass			Sea bream		
	NEFA	TAG	PL	NEFA	TAG	PL
14:0	1.58 ± 0.14	3.10 ± 0.99	1.08 ± 0.38	1.14 ± 0.26	3.43 ± 0.54	1.02 ± 0.10
16:0	18.30 ± 3.32	17.12 ± 1.42	18.91 ± 3.34	21.91 ± 0.81	19.33 ± 1.17	22.19 ± 0.88
16:1(n-7)	2.79 ± 0.49	5.29 ± 1.20	1.48 ± 0.67	2.32 ± 0.26	4.96 ± 0.33	1.32 ± 0.37
18:0	4.03 ± 0.31	3.69 ± 0.18	5.82 ± 0.40	5.98 ± 0.13	4.91 ± 0.26	6.58 ± 0.05
18:1(n-9)	15.19 ± 0.50	19.85 ± 0.57	9.31 ± 0.44	16.59 ± 0.43	23.01 ± 0.69	8.76 ± 0.14
18:2(n-6)	5.60 ± 0.39	9.94 ± 1.39	2.78 ± 0.30	5.76 ± 0.22	8.00 ± 0.16	4.77 ± 0.09
18:3(n-6)	1.43 ± 0.60	1.67 ± 0.13	0.51 ± 0.15	0.57 ± 0.04	0.94 ± 0.30	0.34 ± 0.34
18:3(n-3)	1.01 ± 0.18	7.49 ± 0.17	1.25 ± 0.22	ND	0.07 ± 0.00	0.56 ± 0.02
20:1(n-9)	4.06 ± 0.47	2.92 ± 0.61	0.58 ± 0.42	3.43 ± 0.16	6.41 ± 0.51	1.12 ± 0.11
18:4(n-3)	0.98 ± 0.15	0.65 ± 0.16	0.22 ± 0.05	0.59 ± 0.15	0.67 ± 0.37	0.22 ± 0.04
20:2(n-6)	0.69 ± 0.54	0.20 ± 0.15	ND	ND	0.08 ± 0.00	0.13 ± 0.01
20:3(n-6)	ND	ND	ND	ND	ND	ND
20:4(n-6)	1.48 ± 0.07	2.77 ± 2.19	2.00 ± 0.12	1.39 ± 0.07	0.81 ± 0.03	2.04 ± 0.06
22:1(n-9)	4.36 ± 0.40	7.55 ± 1.78	0.84 ± 0.15	4.79 ± 0.35	7.73 ± 0.46	0.80 ± 0.08
20:5(n-3)	9.45 ± 0.94	8.57 ± 0.68	9.83 ± 1.03	8.49 ± 0.66	7.11 ± 0.11	11.55 ± 0.35
22:4(n-6)	2.75 ± 3.35	0.55 ± 0.07	0.40 ± 0.04	1.01 ± 0.66	0.42 ± 0.06	0.63 ± 0.11
24:1(n-9)	0.71 ± 0.19	1.17 ± 0.11	4.93 ± 0.49	2.55 ± 1.58	1.92 ± 0.32	3.61 ± 0.31
22:5(n-6)	ND	1.91 ± 0.13	1.13 ± 0.12	ND	2.60 ± 0.05	1.82 ± 0.05
22:5(n-3)	2.52 ± 0.59	0.96 ± 0.28	2.07 ± 0.45	3.66 ± 1.34	1.12 ± 0.39	1.07 ± 0.13
22:6(n-3)	25.87 ± 2.27	18.27 ± 2.38	42.76 ± 3.44	21.00 ± 0.73	12.14 ± 0.55	34.71 ± 0.45
Saturated	23.92 ± 2.75	23.91 ± 2.02	25.60 ± 3.52	29.03 ± 0.87	27.68 ± 1.36	29.79 ± 0.80
Monoene	26.83 ± 0.96	36.79 ± 0.95	15.04 ± 1.02	28.01 ± 1.50	44.03 ± 1.10	15.61 ± 0.48
Diene	6.09 ± 0.50	10.06 ± 1.20	2.33 ± 0.47	5.76 ± 0.20	8.06 ± 0.14	4.87 ± 0.07
Triene	1.83 ± 0.79	9.13 ± 0.70	1.76 ± 0.29	0.57 ± 0.04	0.95 ± 0.31	0.45 ± 0.22
PUFA	41.41 ± 2.48	31.96 ± 2.57	57.81 ± 3.82	35.67 ± 1.60	24.19 ± 0.75	52.29 ± 1.07
n-3	38.37 ± 3.32	32.19 ± 3.19	55.24 ± 3.96	33.11 ± 1.56	20.38 ± 0.71	47.56 ± 0.38
n-6	10.04 ± 1.30	14.35 ± 1.33	5.74 ± 0.38	10.18 ± 1.69	12.01 ± 0.54	8.71 ± 0.63
n-3/n-6	4.10 ± 0.59	2.36 ± 0.35	9.76 ± 0.92	3.57 ± 0.55	1.72 ± 0.12	5.59 ± 0.44
nmol·mL^{-1}	2223 ± 32	12260 ± 90[a]		2790 ± 62	9670 ± 58	

[a] n = 12.

Seabass store six times more lipid in their liver per wet weight than sea bream, but they also have large adipose tissue stores, as much as 6.5% body weight depending on the season (G. Brichon, unpublished data). Sea bream have on average 2.8 ± 0.3 g of adipose tissue, roughly 1% of their body mass but, depending on the season, this can be as great as 2.5%. At 80.6 ± 3.5% lipid per tissue wet weight or 874 μmol·g tissue^{-1} it is their major site of storage (Table 4). This value is similar to that found for TAG in adipose tissue of salmonids (800 μmol·g tissue^{-1}; Leger et al. 1981). Subdermal fat is quite reduced in these two species, particularly during late summer (unpublished observation), and is not included here as adipose tissue.

Both species have similar lipid content in their white muscle (3.05 ± 0.35, and 3.17 ± 0.53% wet weight in seabass and sea bream, respectively) or approximately 25 μmol·g tissue^{-1} (Table 5). If we assume that in fish the muscle represents approximately 30–60% body mass (Bone 1978), whole-animal lipid stores in muscle would range from 7500 to 15 000 μmol·kg^{-1} in these fish. Expressed in similar units, the adipose tissue TAG stored in sea bream is 8157 μmol·kg^{-1} and total liver stores are approximately 457 μmol·kg^{-1} in sea bream and about 3333 μmol·kg^{-1} in seabass. Muscle can therefore be a large lipid storage depot when total tissue mass is considered. These numbers are higher in more active fish such as rainbow trout (*Oncorhynchus mykiss*) with muscle lipid content of about 7% wet weight (Henderson and Tocher 1987). Red muscle fibres, although not studied here, are important users of FA during low-intensity prolonged swimming (Moyes et al. 1992; Kiessling and Kiessling 1993). Although lipid content is higher than in white muscle on a per gram basis (40 μmol·g^{-1}) red muscle does not represent a major lipid storage site (about 3 mmol·kg body mass^{-1} in most species; Moyes and West 1994) because of its small size.

Plasma concentration of NEFA is relatively high in both species when compared with other teleosts (1100–2110 nmol·mL^{-1}; Zammit and Newsholme 1979), including levels previously measured for seabass (Zammit

Table 3. Liver FA composition (%) and lipid content per wet weight of TL, TAG, and PL in seabass (n = 5) and sea bream (n = 4).

	Seabass		Sea bream		
	TL[a]	PL	TL	TAG	PL
14:0	1.91 ± 0.20	1.24 ± 0.24	1.78 ± 0.15	3.34 ± 0.69	1.57 ± 0.07
16:0	19.36 ± 0.22	21.06 ± 1.24	20.16 ± 0.89	22.84 ± 1.16	23.92 ± 0.17
16:1(*n*-7)	9.62 ± 0.31	2.84 ± 0.15	5.04 ± 0.44	6.68 ± 0.74	1.34 ± 0.11
18:0	2.98 ± 0.21	9.96 ± 0.71	6.39 ± 0.42	8.58 ± 1.06	9.20 ± 0.70
18:1(*n*-9)	42.47 ± 0.69	12.79 ± 0.68	27.04 ± 2.45	29.97 ± 1.05	9.05 ± 0.23
18:2(*n*-6)	4.70 ± 0.12	2.29 ± 1.98	6.04 ± 0.23	5.86 ± 0.49	4.41 ± 0.23
18:3(*n*-6)	0.09 ± 0.02	3.45 ± 0.25	0.13 ± 0.03	ND	ND
18:3(*n*-3)	0.86 ± 0.11	0.16 ± 0.14	0.98 ± 0.07	0.86 ± 0.00	0.45 ± 0.06
20:1(*n*-9)	4.72 ± 0.24	1.11 ± 0.56	3.16 ± 0.08	2.38 ± 0.79	0.55 ± 0.05
18:4(*n*-3)	0.10 ± 0.00	1.16 ± 1.31	0.91 ± 0.08	2.69 ± 1.39	ND
20:2(*n*-6)	0.27 ± 0.06	0.37 ± 0.05	0.26 ± 0.02	0.66 ± 0.00	0.15 ± 0.00
20:3(*n*-6)	0.24 ± 0.00	0.11 ± 0.00	0.17 ± 0.01	ND	0.31 ± 0.00
20:4(*n*-6)	0.60 ± 0.07	2.71 ± 0.53	1.32 ± 0.14	0.65 ± 0.05	3.11 ± 0.13
22:1(*n*-9)	1.39 ± 0.15	0.48 ± 0.15	4.47 ± 0.80	4.52 ± 0.37	1.11 ± 0.09
20:5(*n*-3)	4.05 ± 0.12	12.05 ± 0.93	5.06 ± 0.43	2.73 ± 0.53	8.85 ± 0.30
22:4(*n*-6)	0.13 ± 0.02	ND	0.31 ± 0.04	ND	ND
24:1(*n*-9)	ND	ND	ND	ND	0.05 ± 0.06
22:5(*n*-6)	0.60 ± 0.02	1.89 ± 0.20	3.11 ± 1.47	5.92 ± 1.25	3.40 ± 1.48
22:5(*n*-3)	ND	ND	4.08 ± 0.29	6.31 ± 0.00	3.35 ± 1.13
22:6(*n*-3)	6.13 ± 0.19	30.78 ± 0.81	17.89 ± 2.74	8.03 ± 1.25	36.95 ± 1.04
Saturated	24.25 ± 0.39	31.83 ± 0.94	28.33 ± 1.07	34.75 ± 1.81	33.99 ± 0.85
Monoene	58.20 ± 0.80	16.88 ± 0.98	39.70 ± 2.63	43.55 ± 0.84	12.10 ± 0.23
Diene	4.96 ± 0.06	2.06 ± 1.11	6.30 ± 0.19	6.02 ± 0.60	4.44 ± 0.20
Triene	1.06 ± 0.18	1.88 ± 1.10	1.25 ± 0.07	0.22 ± 0.22	0.42 ± 0.12
PUFA	11.52 ± 0.40	47.37 ± 1.44	29.62 ± 3.14	20.60 ± 2.18	53.63 ± 0.99
n-3	11.03 ± 0.40	42.38 ± 1.03	26.38 ± 2.86	14.11 ± 3.35	49.52 ± 1.54
n-6	6.46 ± 0.07	8.36 ± 0.48	9.87 ± 0.85	12.10 ± 0.71	8.98 ± 0.72
n-3/*n*-6	1.73 ± 0.03	4.88 ± 0.17	2.74 ± 0.34	1.19 ± 0.29	5.68 ± 0.50
% lipid·wet wt.$^{-1}$	32.8 ± 1.7	1.85 ± 0.07	5.04 ± 0.45	3.3 ± 0.6	1.77 ± 0.12

[a]TL exists as TAG in seabass.

and Newsholme 1979). They have much higher levels than osteichthyans (758 nmol·mL^{-1}, Singer and Ballantyne 1991) and over an order of magnitude higher than most elasmobranchs (150 nmol·mL^{-1}; Zammit and Newsholme 1979) (Table 2). Low plasma NEFA levels in elasmobranchs are not surprising due to the absence of a NEFA carrier protein, while the presence of an albumin-like protein in teleosts allows them to maintain much higher NEFA concentrations (Zammit and Newsholme 1979; Maillou and Nimmo 1993).

If these species are assumed to have the same cardiac output ($\dot{Q}$) (17.6 mL·kg^{-1}·min^{-1} at rest and 52.6 mL·kg^{-1}·min^{-1} at high swimming speed (U_{crit}) in trout; Kiceniuk and Jones 1977) and hematocrit similar to that in other teleosts (chinook salmon (*Oncorhynchus tshawytscha*) at low work rates (23.8%) and at U_{crit} (24.5%); Thorarensen et al. 1993), they can be expected to have NEFA delivery rates higher than most teleosts. Plasma flow ($\dot{Q}(1-\text{Hct})$ or $\dot{Q}_{plasma}$) and NEFA concentration can be used to calculate NEFA delivery rates (McClelland et al. 1994). $\dot{Q}_{plasma}$ in teleosts at rest should be around 13.4 mL·kg^{-1}·min^{-1} and at high work rates around 39.7 mL·kg^{-1}·min^{-1}. NEFA delivery rates in seabass, therefore, might be in the range of 29.8 and 88.3 μmol·kg^{-1}·min^{-1} and in sea bream about 37.4 and 110.8 μmol·kg^{-1}·min^{-1} at rest and at high work rates, respectively. These values can be considered as an underestimate of real in vivo rates because NEFA concentrations will most likely be much higher in both of these species during exercise (Plisetskaya 1980). These rates are, however, about an order of magnitude lower than those found in even the most sedentary mammals (McClelland et al. 1994) but could support 100% of total energy expenditure (from oxygen consumption; Kiceniuk and Jones 1977) at high swimming speeds in trout.

High NEFA concentrations, and possibly flux rate, in these species may be the result of storing lipid outside of muscle in liver and adipose tissue where it must first be mobilized and then transported by the circulation before it can be used by working muscles. Unfortunately, there are no data on NEFA flux rates and little on the storage

of lipid to make any generalizations for all teleosts. Also there is considerable variability among studies in measuring NEFA concentrations in fish owing to different, and often inaccurate, techniques (Singer et al. 1990; Hallaq et al. 1993).

Plasma total TAG in both species fall into the high end of the range of values measured in fish (1000–12 000 nmol·mL^{-1}; Weber and Zwingelstein 1994). Sea bream values are higher than those previously measured in a related species, the red sea bream (*Chrysophrys major*; Sakamoto and Yone 1980). Levels of TAG in fish are much higher than that in the resting fed state of humans (1250 nmol·mL^{-1}) and rats (2000 nmol·mL^{-1}) or even in mammals considered to be endurance adapted (dogs, 1400 nmol·mL^{-1}) (Terjung et al. 1983). These high levels of TAG in fish may be related to the fact that teleosts may rely heavily on circulatory lipids as a fuel source, using glucose and lactate to a much lower extent than mammals. Turnover of glucose in fish (with the exception of tuna and eel) is 1/20 to 1/100 and lactate 1/26 to 1/290 that in mammals of equivalent size (Weber et al. 1986; Weber and Zwingelstein 1994). Even during sustained exercise, estimated oxidation rates of glucose account for only about 10% of aerobic metabolism in trout heart and red muscle (West et al. 1993). Fish have been shown to have a high capacity to oxidize fat, especially in aerobic tissues like red muscle (Moyes et al. 1992; Kiessling and Kiessling 1993). The fish used in this study were not postabsorptive and because fish absorb dietary lipids as NEFA and TAG directly into the bloodstream (Sheridan 1988), this may lead to high plasma levels of both metabolites. TAG may be an important fuel source for exercising fish because, along with PL, it can represent 10–40 times the energy stored in circulatory NEFA (Weber and Zwingelstein 1994). NEFA are thought to be only 5–10% of total plasma lipids (Ballantyne et al. 1993). Plasma in seabass has previously been measured to be 2.3% lipid per wet weight, and 1.6% (70% total plasma lipid) of that was TAG (Brichon 1984). Evidence from mammalian studies show that, at least in the fed state, TAG can supply energy to working muscles in dogs (Terjung et al. 1982). Little work has been done on the potential importance of TAG as a fuel source in fish, but it has been shown in migrating salmon that mobilization of fat fuels is important for long-term swimming, as plasma and whole-body TAG levels decrease over the migration route (Idler and Clemens 1959; French et al. 1983).

FA composition

Dietary lipids not only affect concentration but also FA composition of plasma and tissue lipids (Henderson and Tocher 1987). The FA composition of the diet was reflected in plasma NEFA and TAG as well as liver and adipose tissue TL and TAG (Tables 1, 2, 3, and 4). This suggests that lipids are absorbed in fish as FFA and TAG with little modification to FA composition. Absorption takes place primarily in the anterior intestine and the pyloric caeca, and digestibility increases with degree of unsaturation (those of 18–22 C length being the easiest) and water temperature (Henderson and Tocher 1987). This is reflected in the high proportion of 18:1(*n* - 9) and 22:6(*n* - 3) in all fractions. 22:6(*n* - 3) is thought to be the principal FA in TL of most temperate and tropical fish species, with seawater species being highest in 20- and 22-C unsaturated FA (Henderson and Tocher 1987). Fish are also capable of converting 20:5(*n* - 3) to 22:6(*n* - 3) (Cowey and Sargent 1979) which may explain instances where 20:5(*n* - 3) levels are lower than dietary levels (liver TL in both species and TAG in sea bream) and 22:6(*n* - 3) are higher (muscle and plasma PL in both species). Monoenes, dienes, and PUFA were high in both species, but as expected, trienes were low (Henderson and Tocher 1987).

Both fish have similar PL content in their tissues, about 2% tissue wet weight in liver and 1% in muscle. The pattern of FA in PL, in particular the PUFA fraction, does not follow that of diet, contrary to results found in freshwater fish kept in captivity (Henderson and Tocher 1987). These results are in accordance with Atlantic salmon (*Salmo salar*) where PL FA composition was shown to be independant of dietary FA (Polvi and Ackman 1992). Seabass and sea bream had significantly lower levels of oleate in plasma, liver, and muscle than found in either the diet or in TAG (Tables 1, 2, 3, and 5). As in freshwater fish, PL are richer in PUFA but lower in monoenes than TAG, 22:6(*n* - 3)

Table 4. Adipose FA composition (%) and lipid content per wet weight of adipose tissue in sea bream ($n = 4$).

FA	TL
14:0	1.89 ± 0.21
16:0	13.24 ± 0.39
16:1(*n*-7)	5.95 ± 0.65
18:0	3.69 ± 0.15
18:1(*n*-9)	27.13 ± 1.52
18:2(*n*-6)	5.31 ± 1.65
18:3(*n*-6)	0.05 ± 0.05
18:3(*n*-3)	1.42 ± 0.08
20:1(*n*-9)	7.25 ± 0.22
18:4(*n*-3)	1.68 ± 0.10
20:2(*n*-6)	0.23 ± 0.08
20:3(*n*-6)	ND
20:4(*n*-6)	0.59 ± 0.12
22:1(*n*-9)	8.57 ± 0.34
20:5(*n*-3)	4.82 ± 0.24
22:4(*n*-6)	0.50 ± 0.01
24:1(*n*-9)	ND
22:5(*n*-6)	0.73 ± 0.43
22:5(*n*-3)	3.87 ± 0.41
22:6(*n*-3)	13.11 ± 0.25
Saturated	18.83 ± 0.66
Monoene	48.89 ± 1.78
Diene	5.54 ± 1.61
Triene	1.47 ± 0.12
PUFA	25.29 ± 0.78
n-3	23.21 ± 0.69
n-6	7.41 ± 1.62
n-3/*n*-6	3.84 ± 1.12
% lipid·wet wt.$^{-1}$	80.6 ± 3.5

Table 5. White muscle FA composition (%) and lipid content per wet weight for TL, TAG, and PL in seabass and sea bream (n = 5).

	Seabass			Sea bream		
	TL	TAG	PL	TL	TAG	PL
14:0	2.16 ± 0.32	3.95 ± 0.17	1.16 ± 0.09	2.49 ± 0.24	5.11 ± 0.23	0.97 ± 0.05
16:0	19.27 ± 0.56	19.11 ± 0.84	21.07 ± 0.46	18.90 ± 0.83	20.35 ± 2.24	25.06 ± 0.33
16:1(n-7)	5.56 ± 0.65	7.36 ± 0.57	2.06 ± 0.30	5.95 ± 0.24	10.03 ± 1.00	1.49 ± 0.19
18:0	4.31 ± 0.26	3.11 ± 0.08	6.48 ± 0.17	3.99 ± 0.25	3.66 ± 0.71	7.47 ± 0.30
18:1(n-9)	21.64 ± 1.27	25.70 ± 1.02	12.91 ± 0.45	25.29 ± 1.01	27.67 ± 0.79	11.84 ± 0.22
18:2(n-6)	6.96 ± 0.12	6.39 ± 1.72	3.18 ± 0.10	7.00 ± 0.20	8.24 ± 0.27	4.13 ± 0.10
18:3(n-6)	0.11 ± 0.02	1.82 ± 1.93	0.04 ± 0.00	0.10 ± 0.01	0.08 ± 0.01	ND
18:3(n-3)	1.16 ± 0.03	1.14 ± 0.30	0.50 ± 0.02	1.24 ± 0.06	1.33 ± 0.02	0.50 ± 0.06
20:1(n-9)	5.82 ± 0.62	6.47 ± 1.53	1.93 ± 0.13	4.85 ± 0.21	5.13 ± 0.22	1.36 ± 0.05
18:4(n-3)	1.50 ± 0.53	5.55 ± 4.67	0.44 ± 0.00	1.51 ± 0.09	1.85 ± 0.12	ND
20:2(n-6)	0.26 ± 0.04	0.41 ± 0.06	0.27 ± 0.01	0.25 ± 0.02	0.20 ± 0.05	1.33 ± 1.59
20:3(n-6)	0.08 ± 0.00	0.09 ± 0.01	0.10 ± 0.04	0.11 ± 0.01	0.08 ± 0.01	0.19 ± 0.05
20:4(n-6)	1.17 ± 0.06	0.68 ± 0.03	2.21 ± 0.03	0.97 ± 0.05	0.52 ± 0.06	2.21 ± 0.19
22:1(n-9)	3.67 ± 0.33	3.97 ± 0.96	0.26 ± 0.05	5.07 ± 0.23	6.05 ± 0.18	0.68 ± 0.05
20:5(n-3)	9.00 ± 0.30	8.21 ± 1.13	11.57 ± 0.45	5.90 ± 0.08	4.35 ± 0.35	7.95 ± 0.36
22:4(n-6)	0.37 ± 0.07	0.42 ± 0.02	ND	0.41 ± 0.03	0.70 ± 0.19	ND
24:1(n-9)	ND	ND	0.25 ± 0.02	ND	4.09 ± 0.00	ND
22:5(n-6)	1.50 ± 0.12	1.85 ± 0.14	2.03 ± 0.07	0.86 ± 0.07	ND	ND
22:5(n-3)	0.23 ± 0.00	0.45 ± 0.00	0.81 ± 0.00	2.87 ± 0.12	3.93 ± 0.14	3.99 ± 0.44
22:6(n-3)	19.87 ± 1.61	12.18 ± 0.57	35.63 ± 0.86	18.90 ± 0.92	10.65 ± 0.83	37.64 ± 0.64
Saturated	25.75 ± 0.68	26.17 ± 0.65	28.70 ± 0.41	25.39 ± 0.94	29.13 ± 2.48	33.50 ± 0.33
Monoene	36.69 ± 2.18	43.50 ± 2.79	17.41 ± 0.50	41.15 ± 1.14	49.69 ± 1.76	15.37 ± 0.30
Diene	7.22 ± 0.13	6.80 ± 1.56	3.45 ± 0.09	7.25 ± 0.18	8.40 ± 0.28	4.66 ± 0.46
Triene	1.24 ± 0.05	3.05 ± 1.46	0.58 ± 0.05	1.41 ± 0.06	1.44 ± 0.04	0.57 ± 0.03
PUFA	32.48 ± 1.60	25.66 ± 2.98	51.69 ± 0.29	31.41 ± 0.91	21.54 ± 1.12	51.48 ± 0.73
n-3	30.07 ± 1.61	21.52 ± 1.13	47.86 ± 0.34	28.91 ± 0.87	16.33 ± 1.06	46.08 ± 0.36
n-6	10.28 ± 0.15	11.70 ± 0.50	7.78 ± 0.12	9.64 ± 0.24	13.38 ± 0.42	10.50 ± 0.54
n-3/n-6	2.93 ± 0.19	1.85 ± 0.08	6.17 ± 0.10	3.00 ± 0.07	1.22 ± 0.07	4.44 ± 0.25
% lipid·wet wt.$^{-1}$	3.05 ± 0.35	2.28 ± 0.21	0.77 ± 0.04	3.17 ± 0.53	2.34 ± 0.17	0.83 ± 0.08

being the major PUFA (Henderson and Tocher 1987). This difference most likely reflects the fact that monoenes are preferred as a fuel source and therefore are stored in TAG readily mobilized as an energy source. Maintaining membrane fluidity in the face of high salinity or low temperatures is the primary role played by PUFA in membrane PL. High percentages of 16:0, 18:1, and 22:6 are a reflection of PL preference for even-numbered carbon chains (Stryer 1988) and the preferential incorporation of 22:6(n - 3) by fish into PL (Cowey and Sargent 1979).

Marine lipids are thought to contain more n-3 PUFA leading to higher n-3/n-6 ratios, while freshwater lipids are higher in n-6 FA (Henderson and Tocher 1987). Ratios in these fish ranged from 1.7 in seabass liver TL to 9.76 in seabass plasma PL (Tables 2 and 3). Ratios in PL for both species were slightly lower than those reported previously in marine fish (7.8–18.5) but higher than those found in freshwater fish (1.6–2.0) (Henderson and Tocher 1987; Ballantyne et al. 1993). High n-3 in marine fish has been attributed to high levels of n-3 in their diet, as seen here in a commercial diet (Table 1), and in marine phytoplankton (Ballantyne et al. 1993).

Life-style may be a determining factor in where and how fish species store their lipids. Seabass and sea bream are marine fish living in the Mediterranean with similar life-styles: both species are bottom-dwelling sedentary fish. Both store lipid primarily outside of locomotory muscle. Diet plays the primary role in determining tissue and plasma FA composition. There is a good correlation between dietary FA composition and that found in TAG and NEFA of plasma. The FA composition of the TAG and TL fractions in their storage tissues also closely reflected dietary FA composition, while FA composition of PL did not.

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Mitochondrial DNA and allozyme variation in Atlantic salmon (*Salmo salar*) populations in Wales

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Abstract: Four restriction enzymes were used to assay mitochondrial DNA (mtDNA) variation in Atlantic salmon (*Salmo salar*) in Wales. A total of 122 fish from 22 sites, within three catchments, were analyzed. A further 368 fish from seven of these sites were also investigated at 19–21 allozyme loci. The allozyme and mtDNA data revealed significant genetic differentiation between and within catchments. The allozyme data were as informative as mtDNA restriction fragment length polymorphisms in distinguishing between populations of Atlantic salmon. The similarity between data sets in information content is in contrast with previous studies. The relative value of both techniques to fishery managers is discussed.

Résumé : Quatre enzymes de restriction ont été utilisées pour examiner la variation de l'ADN mitochondrial (ADNmt) chez le saumon atlantique (*Salmo salar*) au pays de Galles. Nous avons analysé au total 122 poissons provenant de 22 sites dans trois bassins versants. Nous avons de plus examiné 368 poissons provenant de sept de ces sites en étudiant 19–21 loci allozymiques. Les données sur les allozymes et l'ADNmt ont révélé une différenciation génétique importante entre les bassins et à l'intérieur de ces bassins. Les données sur les allozymes ont été aussi utiles que celles sur le polymorphisme de longueur des fragments de restriction de l'ADNmt pour distinguer les différentes populations de saumon Atlantique. La similarité entre les séries de données en ce qui concerne l'information obtenue se différencie des résultats des études antérieures. Nous analysons la valeur relative des deux techniques pour les gestionnaires des pêches.
[Traduit par la Rédaction]

Introduction

The demonstration of significant differences in allozyme frequencies between regions has greatly facilitated the management of some salmonid species, for example the mixed stock fishery of Pacific salmon (*Oncorhynchus* spp.) (Shaklee et al. 1991). Allozyme variation has been described in Atlantic salmon (*Salmo salar*) (see Jordan et al. 1992 and references therein), but only 8% of the loci so far investigated have proved useful in distinguishing between populations (Davidson et al. 1989). Thus, it is impossible to estimate the proportional contribution of populations from individual countries to the high-seas fishery on the basis of the available data. In order to expand the genetical information base for Atlantic salmon, mitochondrial DNA (mtDNA) restriction fragment length polymorphisms (RFLPs) have also been investigated (Birt et al. 1986, 1991; Hovey et al. 1989; Palva et al. 1989; Bermingham et al. 1991; King et al. 1993).

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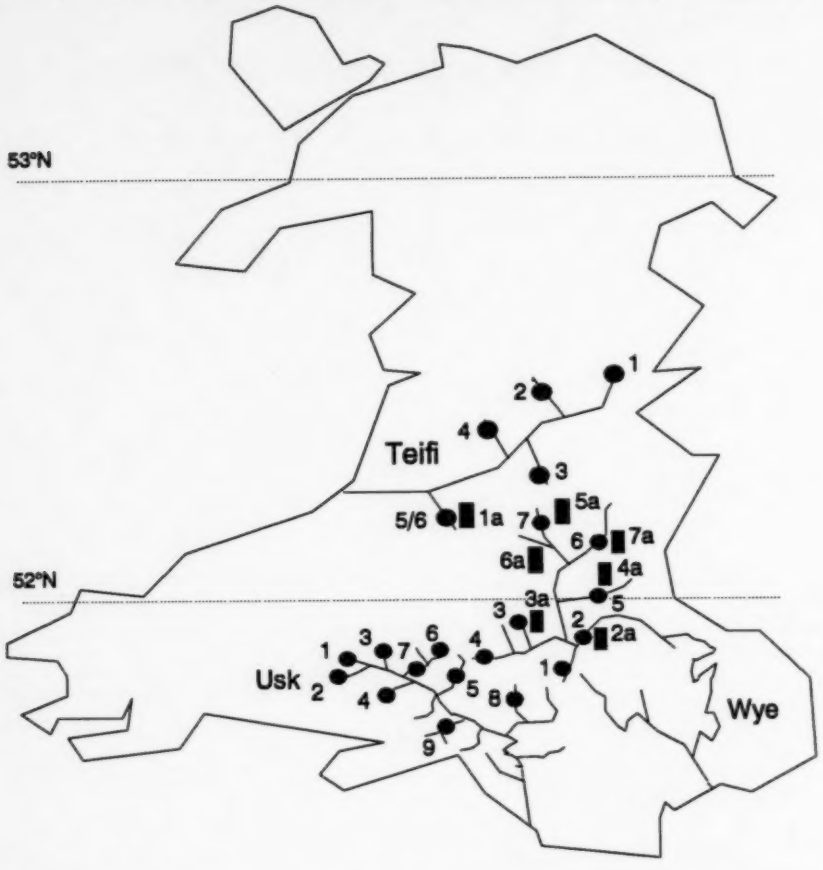
The objective of this study is to describe genetic variation in Welsh populations of Atlantic salmon, for which almost no data are available, using allozymes and mtDNA RFLPs. From a conservation aspect, baseline genetic surveys allow managers to monitor the genetic effects, if any, of subsequent propagation measures. We compare the results from allozyme and mtDNA analyses and assess the relative value of both techniques to fishery managers.

Materials and methods

A total of 368 fish were collected for allozyme analysis from six sites distributed throughout the Wye catchment in October 1990 and a single site from the Teifi catchment in February 1992. Second-year parr were collected for mtDNA analysis from 20 sites from the Usk, Wye, and Teifi catchments in October–November 1990 and February 1992 (Fig. 1). Fish were collected by electrofishing into the prevailing current and killed using Benzocaine. Tissue samples for allozyme analysis were frozen in liquid nitrogen at the river bank. Standard horizontal starch gel (12%) electrophoresis was carried out to investigate the products of *AAT-3**, *AAT-4**, *EST-1**, *EST-2**, *GPI-1**, *GPI-2**, *IDDH-1**, *IDDH-2**, *IDHP-2**, *IDHP-3**, *LDH-1**, *LDH-2**, *LDH-3**, *LDH-4**, *MDH-1**, *MDH-2**, *MDH-3**, *MDH-4**, *MEP-1**, *MEP-2**, and *SOD-1**. The stain recipes and buffers used were those described by McElligott (1987).

Fish used for mtDNA analysis were dissected at the river bank; the heart, liver, and female gonads used, as

Fig. 1. Distribution of the sampling sites. Circles represent the mtDNA sampling sites, rectangles represent the allozyme sampling sites, and the numbers correspond to Table 1.



the source of mtDNA, were immediately immersed in liquid nitrogen. Alternatively, fish were brought back to the laboratory on wet ice and mtDNA extracted from fresh tissue. At least 0.2 g of tissue was required for the extraction of sufficient quantities of mtDNA. The extraction procedure used was a modification of that described by Lansman et al. (1981). All mtDNA samples were digested with *Ava*II, *Hae*III, *Hin*fI, and *Mbo*I. Some samples from the Usk and Wye were also digested with *Dde*I and *Msp*I. All digestions were carried out overnight according to the manufacturer's (GIBCO) instructions. Restriction fragments were end-labelled with α^{32}P, using the Klenow fragment of DNA polymerase I and resolved on 5% polyacrylamide gels. A ϕX174 *Hin*fI ladder (Stratagene) was used as a molecular weight marker. Gels were dried under vacuum for 2 h at 80°C. Autoradiography was generally carried out for 2 h to 3 days without intensifying screens.

Statistical analyses of allozyme data were carried out using the computer packages BIOSYS-1 (Swofford and Selander 1981) and a slightly modified version of DIPLO (Weir 1990). Chi-squared (χ^2) tests for heterogeneity in allele frequency between populations were summed for each variable locus, with the degrees of freedom being additive (see McElligott and Cross 1991). The within-catchment analysis did not include the *IDDH-1** and *IDDH-2** loci. Statistical analyses of mtDNA data were performed using modified HAPLO (Weir 1990) and REAP computer packages (McElroy et al. 1991).

Results

Allozymes

All populations were screened at 19–21 loci. The variable number was due to initial staining problems observed with *IDDH-1** and *IDDH-2**. A locus was considered polymorphic (Table 1) if the frequency of the common allele did not exceed 0.95. The percentage of polymorphic loci (excluding *IDDH-1** and *IDDH-2**) was 15.8%. The mean unbiased heterozygosity value (Nei 1978), excluding *IDDH-1** and *IDDH-2**, over all the populations investigated was 5.8%. The values of mean heterozygosity vary considerably, with the Wye catchment values ranging from 4.7 to 6.2%. The value for the Teifi was 7.2%.

Within the Wye catchment, 20% of the χ^2 pairwise population comparisons revealed significant ($P < 0.05$) allele frequency heterogeneity, when corrected for type I statistical

Table 1. Allele frequencies at five polymorphic loci in salmon sampled from seven sites (ND, no data).

Allele	Teifi 1a	Ross 2a	Garth Dulas 3a	Edw 4a	Glan-Gwy 5a	Marteg 6a	Ithon 7a
*AAT-4**							
100	0.615	0.500	0.510	0.589	0.538	0.700	0.500
74	0.385	0.500	0.490	0.411	0.462	0.300	0.500
n	48	44	51	56	40	60	38
*IDHP-2**							
116	0.250	0.065	0.086	0.071	0.024	0.008	0.031
100	0.750	0.935	0.914	0.929	0.976	0.992	0.969
n	48	46	35	56	41	60	49
*MEP-2**							
115	0.448	0.443	0.483	0.298	0.315	0.339	0.429
100	0.512	0.557	0.517	0.702	0.685	0.661	0.571
n	40	53	30	57	54	59	42
*IDDH-2**							
100	0.625	ND	ND	0.663	0.648	0.500	0.656
72	0.375	ND	ND	0.337	0.352	0.500	0.334
n	48			40	27	63	45
*IDDH-1**							
100	0.750	ND	ND	0.887	0.870	0.921	0.878
28	0.250	ND	ND	0.112	0.130	0.079	0.122
n	48			40	27	63	45

errors using the Bonferroni procedure (Rice 1989). The pooled F_{IS} (−0.022) and f (Cockerham 1969) (−0.046) values were small and negative. Cockerham's (1969) F statistics (f, F, and theta (θ)) were calculated, as they are recommended for sample sizes less than 100 (Black and Krafsur 1985). Cockerham's θ coefficient was also calculated for mtDNA and this statistic allows direct comparison of the two data sets. When F_{IS} and f were tested for significance (see Waples 1987), neither value deviated significantly from the Hardy–Weinberg expectation. The F_{IT} (−0.001) and F (−0.029) values were also small, which suggests a lack of inbreeding within the catchment. The F_{ST} and θ values were 0.020 and 0.016, respectively. A χ^2 test was used to test the statistical significance of the F_{ST} value (see Waples 1987 and Jordan et al. 1992 for more details; $P < 0.05$). This value indicated a significant level of structuring between populations within the Wye catchment. The extent of structuring between all sites in both catchments was also measured. The F_{ST} (0.029) and θ (0.025) values were both significant ($P < 0.01$).

mtDNA

Composite haplotypes were available for 126 Atlantic salmon from 21 sites dispersed over the Usk, Teifi, and Wye catchments. Two genotypes were observed for each of the restriction enzymes *Ava*II, *Hae*III, *Hin*fI, and *Mbo*I. *Msp*I was completely invariant in the Wye and Usk populations and the Teifi was not screened with this enzyme. Although variants were observed using *Dde*I, limited quantities of mtDNA precluded a more comprehensive investigation with this enzyme. The restriction patterns for all the commonly used enzymes were similar to those described elsewhere (Hovey et al. 1989; Palva et al. 1989; Knox and Verspoor 1991; King et al. 1993).

Ten composite mtDNA haplotypes were observed with the four commonly used enzymes (Table 2). Haplotypes 5 and 8 were restricted to a single individual. The percent nucleotide diversity estimates (Nei 1987), calculated on the basis of divergence between haplotypes and their frequency within the population, varied between catchments. The Usk had the lowest level of mtDNA nucleotide diversity at 0.07%, while the Teifi (0.13%) and Wye (0.15%) had considerably higher levels of diversity. The θ values (Table 3), which measure the extent of haplotype structuring, for each of the three catchments revealed that mtDNA variation was significantly partitioned in two of the three catchments investigated. The total level of structuring between all the sites from the three catchments is given in Table 3.

Discussion

The average heterozygosity determined in this study, based on 19 allozyme loci, is 5.8%. This falls well within the range of values seen for Atlantic salmon elsewhere (Ståhf 1987; Sanchez et al. 1991; Jordan et al. 1992). However, electrophoretic studies of Atlantic salmon are generally concentrated on the same five or six informative loci; thus, estimates of mean heterozygosity in studies on Atlantic salmon can be misleading.

Average haplotype diversity estimates (Nei 1987) for populations varied between 0 and 0.85. This large range

Table 2. Composite genotype frequencies at each of the sampling sites investigated.

Site no. (sample size)	Teifi						Usk									Wye						
	1 (8)	2 (2)	3 (6)	4 (8)	5 (8)	6 (4)	1 (2)	2 (1)	3 (8)	4 (10)	5 (3)	6 (7)	7 (6)	8 (5)	9 (8)	1 (5)	2 (3)	3 (6)	4 (7)	5 (6)	6 (7)	7 (2)
mtDNA haplotype																						
1 AAAA[a]	0.375	1.000	0.333	1.000	0.750	0.750	0.500	—	0.375	0.500	0.333	0.571	0.833	0.600	0.750	0.600	1.000	0.400	0.714	0.333	0.143	—
2 AAAB	—	—	—	—	0.125	—	—	—	—	—	—	—	—	—	0.125	—	—	—	0.143	—	—	0.500
3 AABA	0.125	—	0.167	—	—	—	—	—	—	—	—	—	—	0.400	—	—	—	—	—	—	—	—
4 ABAA	—	—	0.167	—	—	—	0.500	1.000	0.500	0.400	0.333	0.143	0.167	—	0.125	0.400	—	0.400	—	0.167	0.285	—
5 BAAA	—	—	0.167	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
6 AABB	0.125	—	—	—	—	—	—	—	—	0.100	—	—	—	—	—	—	—	—	—	0.167	0.143	—
7 ABAB	—	—	—	—	—	—	—	—	—	—	—	0.286	—	—	—	—	—	—	—	—	—	—
8 BAAB	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.500
9 BABA	0.125	—	0.166	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.429	—
10 BABB	0.250	—	—	—	0.125	0.250	—	—	0.125	—	0.333	—	—	—	—	—	—	0.200	0.143	0.333	—	—

[a]Restriction enzyme order in composite genotype was *Hin*fI, *Mbo*I, *Ava*II, and *Hae*III.

Table 3. Extent of mtDNA differentiation using θ (***P < 0.001).

	Teifi	Usk	Wye	Between sites
*Hin*fI	−0.018	0.135	−0.019	0.083
*Mbo*I	0.007	0.047	−0.013	0.067
*Ava*II	0.244	0.006	0.236	0.168
*Hae*III	0.022	−0.010	0.196	0.099
Total	0.097***	0.032	0.103***	0.106***
	(5)	(15)	(4)	(4)

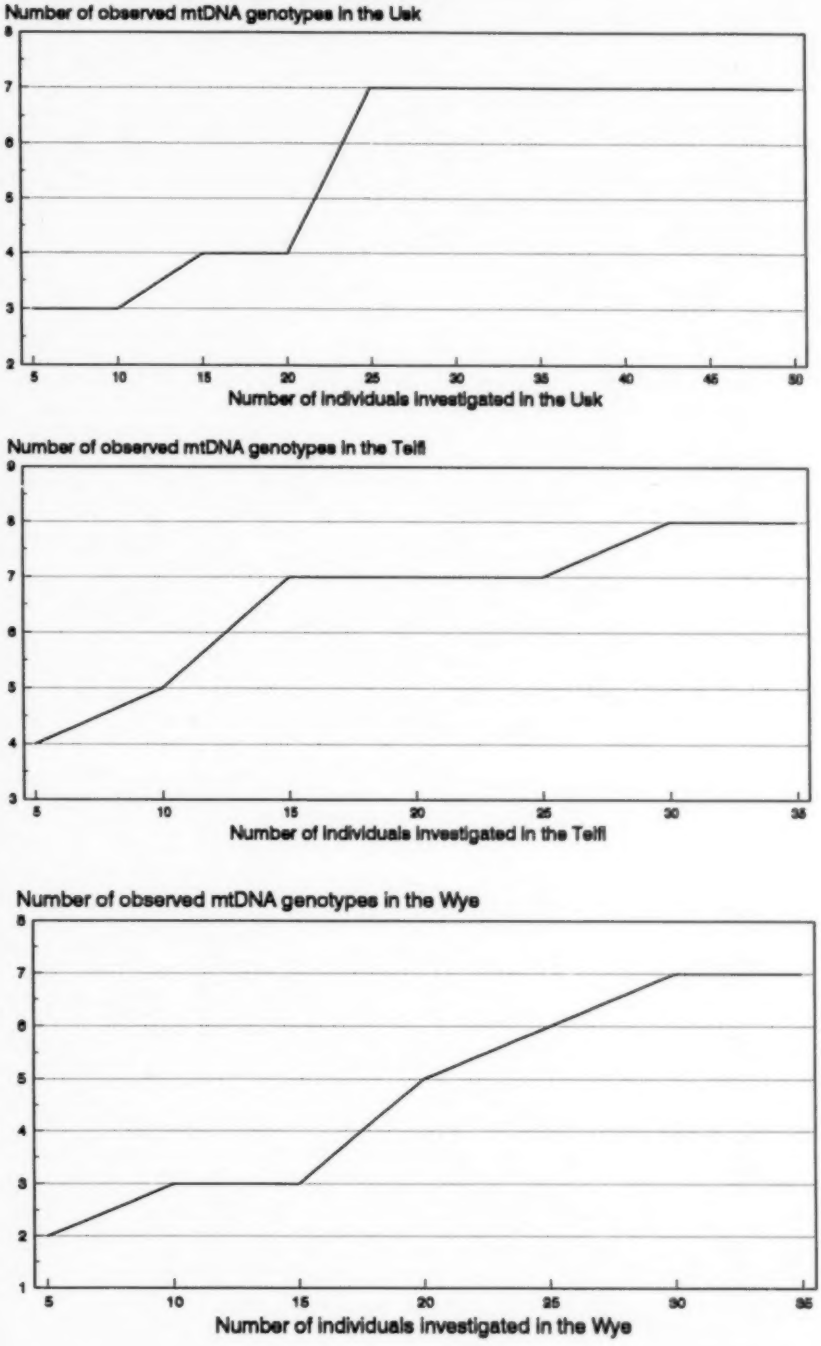
Note: The numbers in parentheses refer to the effective number of genetic migrants (N_{em}) calculated according to Birky et al. (1983).

in observed values was probably due to the low average sample size (n = 6), causing rare genotypes to be absent. When the sites are grouped within their own catchments, the range in values was much smaller (0.07–0.15).

The allozyme data set revealed significant differences between populations of Atlantic salmon within and between catchments. The absolute number of genetic migrants, or strays (N_{em}) was calculated to be 8 or 10 depending on whether it was based on the F_{ST} or θ coefficient. The model on which the N_{em} calculation is made assumes an infinite number of populations, an equal likelihood of migration between any two populations, and genetic equilibrium. In Atlantic salmon, these assumptions are unrealistic. The relatively high value of gene exchange observed, in population genetic terms, would lead to a loss of allele and haplotype differentiation. However, if the populations investigated are not in genetic equilibrium, the N_{em} figure may represent an upper limit with levels of gene flow ranging between 8 and 0. However, from the management viewpoint, the value obtained does suggest that there are restrictions to gene flow between populations and supports a homing hypothesis for Atlantic salmon within catchments. These data are in agreement with previous genetic studies on Atlantic salmon by Ståhl (1987), McElligott and Cross (1991), Verspoor et al. (1991), and Jordan et al. (1992). Estimates of salmon straying rates (N_{em}) derived from genetic data include 2% (Thorpe 1988), 3% (Altukhov and Salmenkova 1991), and 5% (Verspoor et al. 1991). These values are generally below those calculated from physical tagging data (2–7%), with some farmed fish showing straying rates of up to 18% (Thorpe 1988). Tagging is likely to overestimate interpopulation migration because some tagged fish will probably not spawn successfully and thus fail to generate effective migrants. The estimates of N_{em}, derived from mtDNA, were also low from a management perspective (Table 3). Therefore, results from both data sets suggested that the Teifi, Usk, and Wye catchments should be managed as multistock Atlantic salmon fisheries.

Reduced levels of genetic variability are often associated with artificially reared fish (Cross and King 1983; Vuorinen 1984; Verspoor 1988; Hindar et al. 1991). The reduced effective population size (N_e) for mtDNA increases its sensitivity to inbreeding and bottleneck events and makes it ideal for investigating the genetic effects of hatchery rearing.

Fig. 2. Number of observed mtDNA haplotypes as a function of the number of individuals investigated in the three catchments.


Number of observed mtDNA genotypes in the Usk
Number of individuals investigated in the Usk
Number of observed mtDNA genotypes in the Teifi
Number of individuals investigated in the Teifi
Number of observed mtDNA genotypes in the Wye
Number of individuals investigated in the Wye

The three catchments investigated have hatcheries. These hatchery-reared fish are used to supplement runs of wild Atlantic salmon. The level of mtDNA variation within the Usk was approximately half that of the other two catchments investigated. This may be the result of one or more natural historical bottlenecks. However, an alternative explanation, considering the Usk is intermediate both in terms of its catch statistics and physical size, may be past or present

hatchery practices on the catchment. The Usk was also identified as having the lowest level of within-catchment differentiation. This may reflect a gradual breakdown of reproductive barriers due to the annual large-scale releases of hatchery-reared parr and smolts into the catchment.

An important question that has yet to be addressed by fishery managers handling mtDNA haplotype frequency data is the minimum sample size required to reflect accurately the haplotype frequencies in the population. Initially, mtDNA studies were applied to terrestrial species with a limited capacity for gene flow (Avise 1989). These surveys revealed that individual mtDNA genotypes are often confined to single populations. However, when applied to marine species, a widespread distribution of common genotypes is usually observed (Graves et al. 1984, 1992; Smith et al. 1989). Atlantic salmon mtDNA genotypes appear to be widely distributed between populations. Thus, the identification of significant differences in mtDNA genotypes between populations requires larger sample sizes than those needed in the early studies of terrestrial species. Our data revealed that 25–30 individuals were required to identify all genotypes present (Fig. 2). This result suggests that the diversity estimates for the individual sites may underestimate the true values. In contrast, estimates at the catchment level appeared largely valid.

Although a sample of 25–30 individuals is adequate for investigating mtDNA diversity, the number of individuals required to differentiate between closely related populations will be greater. This is apparent when the mtDNA molecule is considered as a single locus, with the composite genotypes equivalent to alleles. Ten mitochondrial alleles were observed in the present study; thus the potential informative value of the molecule is readily appreciated. However, to accurately assess these allele frequencies, some of which occur at very low frequencies, larger sample sizes will be required. Sampling error could have biased the between-site divergence values in the present survey. However, the identification of mtDNA structuring was in agreement with the allozyme data set. The ultracentrifugation technique used to extract mtDNA in this study cannot practically be used to examine large numbers of fish. However, rapid PCR-based RFLP analyses of variable regions of the molecule should make such investigations feasible (Hall 1992; Cronin et al. 1993; O'Connell 1993). The PCR approach has the added benefit that it can be used in conjunction with a nonlethal sampling regime.

The use of mtDNA RFLP analysis is now becoming routine for Atlantic salmon (Hovey et al. 1989; Palva et al. 1989; Bermingham et al. 1991; Knox and Verspoor 1991; King et al. 1993). In the present study, individuals sampled for both data sets were collected from the same populations. This allows the relative value of mtDNA and allozymes, for determining Atlantic salmon population structure, to be assessed. At particular sites, patterns of variability from both data sets were not correlated. In contrast with most previous studies (Avise 1986; Ward et al. 1989), our results suggested that allozymes are at least as useful as mtDNA in describing population differentiation; this is consistent with Ferguson et al. (1991) who also observed that allozymes may be more informative than mtDNA. The increased sensitivity of mtDNA to fluctuations in the population size, leading to depressed levels of variability, may mean that the molecule will not generally prove more informative than allozymes in salmonids.

One potential genetic tag that could be used for identification of introduced fish to the area is the *MDH-3/4** locus. This locus was completely invariant in all the populations investigated, in contrast with results from Scottish and Irish populations of Atlantic salmon (McElligott and Cross 1991; Jordan et al. 1992). Therefore, introductions of selected variants at this locus would be readily identifiable to fishery managers in Wales. However, from a conservation aspect, managers should only consider such introductions as a last resort.

In conclusion, mtDNA and allozyme analysis revealed a significant degree of genetic isolation between Atlantic salmon populations, both within and between catchments. From the viewpoint of genetic management, the mtDNA data suggested that the existing propagation measures may not be sufficient to offset a loss of mtDNA diversity in all catchments. The study shows allozymes to be at least as informative as mtDNA in discriminating between Atlantic salmon populations in the region. This suggests that alternative DNA approaches such as satellite DNA should also be calibrated against existing data sets so that their relative power in discriminating between populations can be assessed.

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Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*)

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Abstract: Although mitochondrial introgression between taxa has been increasingly documented, interspecific replacement of mtDNA is rare, particularly when the donor species is absent. We document evidence for a population of brook trout (*Salvelinus fontinalis*) in which all individuals possess the mitochondrial genome of Arctic char (*S. alpinus*) despite the present-day absence of the latter species in the watershed where the population is located. The mitochondrial genotype of 48 brook trout from Lake Alain (Québec) was characterized by RFLP analysis performed over the entire mtDNA molecule and/or a 2.5-kb PCR-amplified segment of the ND-5/6 region. Although the fish examined were morphologically indistinguishable from typical brook trout and homozygous for the diagnostic alleles characteristic of brook trout, the mtDNA of all individuals was identical to the Québec Arctic char haplotype. Together, these results indicate that the mtDNA haplotype observed in Lake Alain brook trout has resulted from ancient introgression with Arctic char rather than ancestral polymorphism or convergent evolution. They also demonstrate that introgressive hybridization between those two species can have significant and long-term effects on their genetic composition.

Résumé : On rapporte de plus en plus l'introgression mitochondriale entre des taxons, mais l'échange d'ADNmt entre deux espèces est rare, notamment lorsque l'espèce donneuse est absente. Nous présentons une population d'Ombles de fontaine (*Salvelinus fontinalis*) dont tous les individus possèdent le génome mitochondrial de l'Omble chevalier (*S. alpinus*) sans que cette dernière espèce soit présente actuellement dans le bassin où la population d'Ombles de fontaine a été localisée. Le génome mitochondrial de 48 Ombles de fontaine provenant du lac Alain (Québec) a été caractérisé par une analyse des polymorphismes au niveau de toute la molécule d'ADNmt et/ou d'un segment de 2,5 kb de la région ND-5/6 amplifié par la PCR. Les poissons examinés étaient indifférenciables morphologiquement d'un omble de fontaine typique et étaient homozygotes pour les caractères allèles de diagnostic de l'Omble de fontaine, mais l'ADNmt de tous les individus était identique à l'haplotype d'ADNmt de l'Omble chevalier du Québec. Tous ces résultats indiquent que l'haplotype d'ADNmt observé chez l'Omble de fontaine du lac Alain provient d'une ancienne introgression avec l'Omble chevalier plutôt que d'un polymorphisme ancestral ou d'une convergence. Ces résultats montrent également qu'une hybridation introgressive entre ces deux espèces peut avoir des effets importants et à long terme sur leur composition génétique.

[Traduit par la Rédaction]

Introduction

There is little consensus regarding the evolutionary significance of hybridization and introgression, although there is increasing evidence for their occurrence. Traditionally, the transfer of genes across species boundaries has been thought to have little or no evolutionary importance (Mayr 1963; Heiser 1973). The main argument supporting this view is that introgressive hybridization among animal taxa is rare and that documented cases leading to persistent,

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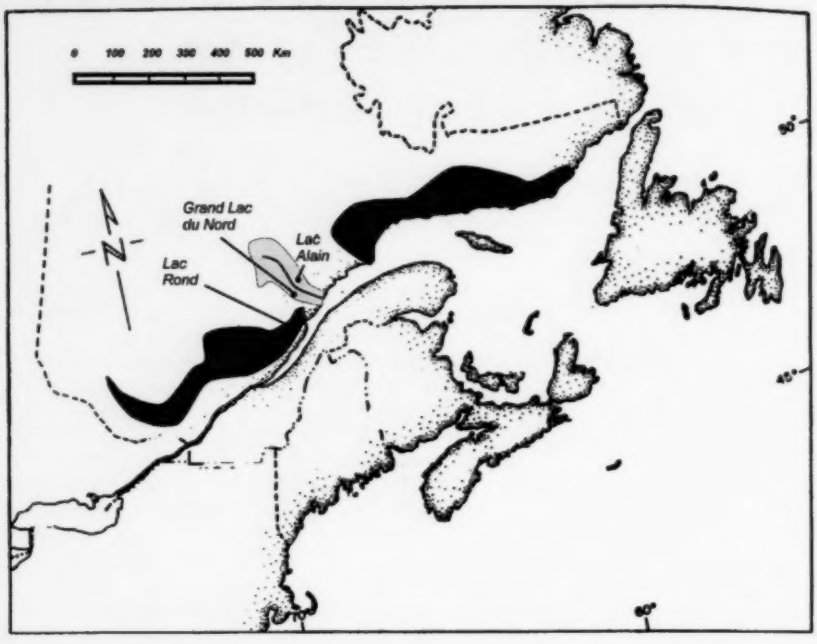
Fig. 1. Location map of Lake Alain, Lac Rond, and Grand Lac du Nord showing present-day distribution of land-locked Arctic char populations on the north shore of the St. Lawrence River, as well as the limits of the Portneuf River watershed.



long-term incorporation of one species' genes into another are even rarer. Recent molecular and ecological studies, however, support the prevalence of introgressive hybridization in several species complexes and argue that even rare introgressive events may be more important than mutation as a source of new genetic variability within taxa (reviewed in Arnold 1992).

Introgressive hybridization could be particularly important in fish, which, as a group, appear to hybridize fairly readily (Hubbs 1955). Hybridization beyond the first generation has been reported for many species (reviewed in Dowling et al. 1989; Dowling and Hoeh 1991; Verspoor and Hammar 1991; Carmichael et al. 1993), suggesting the potential for introgression.

One well-documented case of potential interspecific introgression is the natural hybridization between Arctic char (*Salvelinus alpinus*) and brook trout (*Salvelinus fontinalis*) reported by Hammar et al. (1991). Protein electrophoresis detected repeated hybridization between these species in northern Labrador. Furthermore, isozyme phenotypes suggested the presence of second-generation hybrids and/or backcrosses to both species. However, there was no evidence of persistent incorporation of genes from one species into the other, precluding any determination of the evolutionary impact of hybridization for these species.

In this paper, we report the fixation of Arctic char mitochondrial DNA (mtDNA) in an allopatric population of brook trout from an inland lake in Québec, outside the present-day contact zone of the two species. This shows that introgressive hybridization as described by Hammar et al. (1991) can have significant and long-term effects on the genetic composition of brook trout. This case of mitochondrial introgression represents one of the very few documentations of complete interspecific replacement of mitochondrial genome and the first known occurrence in salmonid fishes.

Material and methods

Sample description and location

Brook trout were collected from Lake Alain (48°48′00″, 69°35′30″) and Grand Lac du Nord (48°42′00″, 69°40′00″), which are in the Portneuf River system (Fig. 1), in the context of a macrogeographic survey of brook trout in eastern North America (R.G. Danzmann et al., unpublished data). Sixteen fish were collected from Lake Alain in August 1992, and 32 additional fish were collected between January and July 1993. Arctic char does not occur in Lake Alain or its river system and the nearest reported population is approximately 50 km away (Dumont 1982; M. Brault, Ministère du Loisir, de la Chasse et de la Pêche, Québec, personal communication). Arctic char were collected from Lake Rond (48°15′00″, 70°37′30″) and obtained from a local pisciculture. Arctic char obtained from the pisciculture were of Fraser River (Labrador) origin. Liver was sampled from each fish and either processed immediately or stored at −80°C for later analysis.

Morphological identification

Upper and lower gill rakers were counted on the first left gill arch, and external features which are classically used to distinguish Arctic char and brook trout were recorded on all freshly killed fish (Scott and Crossman 1974). The

diagnostic features examined were tail shape (square versus forked), coloration of lower fins (presence/absence of a black stripe following the white border), presence/absence of vermiculations on the back, caudal, and dorsal fins, and presence/absence of red spots with a blue halo on the sides of the body.

Allozyme analysis

Protein electrophoresis was carried out on cellulose acetate using liver tissue homogenates as described by Hebert and Beaton (1989). Fish were screened at several loci known to be polymorphic for Arctic char and brook trout (Hammar et al. 1991). Four diagnostic enzymes representing six loci were utilized to distinguish brook trout from Arctic char and included isocitrate dehydrogenase (IDH, 1.1.1.42), lactate dehydrogenase (LDH, 1.1.1.27), sorbitol dehydrogenase (SDH, 1.1.1.14), and superoxide dismutase (SOD, 1.15.1.1). Loci were designated as recommended by Shaklee et al. (1989). All enzymes examined were resolved by using a Tris glycine buffer system of pH 8.5 and the staining recipes outlined by Hebert and Beaton (1989). Allelles were identified by their relative electrophoretic mobilities as measured from the gels. The most common allelle in the brook trout population (Grand Lac du Nord) was designated a standard mobility of 100.

Characterization of mtDNA

For the first 16 individuals analyzed from Lake Alain, mtDNA was extracted and purified from fresh liver by the rapid isolation method of Chapman and Powers (1984) with modifications of Danzmann et al. (1991*a*). Intact mtDNA was digested with eight restriction enzymes (*Acc*I, *Bam*HI, *Bcl*I, *Bst*EII, *Nco*I, *Nhe*I, *Pst*I, *Xba*I). Digested mtDNA fragments were separated on 0.8% agarose gel run overnight at 30 V, visualized by UV irradiation after ethidium bromide staining, and photographed. All of these enzymes provided diagnostic fragment patterns between Arctic char and brook trout throughout their distributions (Grewe et al. 1990; Danzmann et al. 1991*a*, 1991*b*; Ferguson et al. 1991; Bernatchez and Danzmann 1993; Wilson and Hebert 1993; C. Wilson, unpublished results). Digested samples were subsequently compared against mtDNA digests from Québec Arctic char (Lake Rond) and brook trout (Grand Lac du Nord).

Total DNA was extracted from the remaining 32 individuals of Lake Alain as described previously by Bernatchez et al. (1992). A 2.5-kb fragment of the mitochondrial genome encompassing the ND-5/6 region was amplified by the polymerase chain reaction (PCR) with the primers published by Cronin et al. (1993). PCR reactions were those described by these authors and the amplification conditions consisted of a first denaturation step of 95°C for 1 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 3 min. Amplified DNA was digested with three additional restriction enzymes (*Ava*I, *Hae*III, and *Hin*cII) which generated diagnostic fragment patterns in the ND-5/6 segment between Arctic char and brook trout. The DNA fragments were separated on 1.2% agarose gels, run for 5 h at 85 V, stained with ethidium bromide, and photographed with polaroid under UV light.

Table 1. Allele frequencies at diagnostic loci for Arctic char (SA) and brook trout (SF). Introgressed char and trout are from Lake Alain and are represented as LA. The number of fish examined at each locus is given in parentheses.

Locus and allele	SA (20)	LA (30)	SF (24)
*IDH-3,4**			
(*100/100*)	0	1.00	1.00
(*130/130*)	1.00	0	0
*LDH-3**			
($0^a/0^a$)	0	1.00	1.00
(*100/100*)	1.00	0	0
*SDH-1,2**			
(*100/100*)	0	1.00	1.00
(*40/40*)	1.00	0	0
*SOD**			
(*100/100*)	0	1.00	1.00
(*260/260*)	1.00	0	0

[a]The allele present in *S. alpinus* was assigned a value of *100*, as the *S. fontinalis* allele remained at the origin.

Results

Morphological identification

Based on gill raker counts and external features, all Lake Alain fish collected were unambigiously identified as brook trout. Gill raker counts (LGR range 6-7, UGR range 10–12, total range 15–19) were typical of brook trout populations when compared with overall ranges reported for that species (UGR range 4–7, LGR range 10–15, total range 14–22) and for Arctic char (UGR range 7–13, LGR range 12–19, total range 19–32). All 48 individuals analyzed had a square tail, vermiculated patterns on the back, dorsal, and caudal fins, a white border followed by a black stripe on lower fins, and red spots with a blue halo on the body sides. These morphological characteristics are all unambiguously typical of brook trout.

Allozymes

Identification of char and trout from Lake Alain (LA) was based on diagnostic fixed alleles between *S. alpinus* (SA) and *S. fontinalis* (SF) at the *IDH*-3,4*, *LDH*-3*, *SDH*-1,2*, and *SOD** loci (Table 1). Alleles at all loci in the char and trout from Lake Alain were found to be identical to those characteristic of *S. fontinalis*, with no evidence of shared alleles with *S. alpinus*.

mtDNA characterization

Restriction analysis performed either over the entire molecule or on the ND-5/6 segment clearly showed that 100% of the 48 individuals characterized possessed only mtDNA of Arctic char origin (Table 2, Fig. 2). Only one mtDNA genotype was observed in Lake Alain which matched the genotype of *S. alpinus* from Lac Rond, which is also the dominant genotype observed among many populations of

Table 2. List of mtDNA fragments generated by restriction digests of Québec brook trout, Lake Rond (SA), Lake Alain fish (LA), and Québec brook trout, Grand Lac du Nord (SF). Presence (1) or absence (0) of different fragment patterns is indicated. Arctic char (SA) samples have fragment patterns typical of Arctic char from southeastern Québec. Brook trout samples (SF) are also representative. The number of fish examined is given in parentheses.

Enzyme	Fragment sizes						SA	LA	SF
Total mtDNA digests							(25)	(16)	(24)
*Acc*I	6450	5040	2080	1700	1000	500	1	1	0
	8600	3420	2980	1700	480	330	0	0	1
*Bam*HI	15520	1270					1	1	0
	14350	2450					0	0	1
*Bcl*I	8100	7850	1000				1	1	0
	8100	4060	3610	1020			0	0	1
*Bst*EII	9350	4500	2380	580			1	1	0
	7090	4850	4250	560			0	0	1
*Nco*I	7200	6700	1800	1180			1	1	0
	8980	7720	1180				0	0	1
*Nhe*I	7470	7470	1870				1	1	0
	7700	6400	3300				0	0	1
*Pst*I	16800						1	1	0
	16490	310					0	0	1
*Xba*I	13600	3200					1	1	0
	6150	5190	3200	2420			0	0	1
ND-5/6 segment							(25)	(32)	(24)
*Ava*I	2000	520					1	1	0
	1450	520	520				0	0	1
*Hae*III	600	600	560	350	350		1	1	0
	730	730	380	380	220		0	0	1
*Hin*cII	1050	900	560				1	1	0
	1050	950	520				0	0	1

Fig. 2. Photograph of an ethidium bromide stained 1.2% agarose gel showing restriction fragments produced by digestions of the ND-5/6 amplified segment with *Ava*I (lanes *b* to *i*) and *Hae*III (lanes *j* to *q*). For both enzymes, the first two lanes correspond to individuals from Lake Alain and the next two of Arctic char from Lake Rond (Québec). The four others are of brook trout from Grand Lac du Nord (Québec). Lanes *a* and *r* are lambda phage cut with *Hin*dIII and *Eco*RI (double digest).

Arctic char in eastern North America (C. Wilson, unpublished data). This genotype was also similar to the dominant genotype reported for Icelandic char (Danzmann et al. 1991*b*), with the exception of the fragment pattern for *Pst*I. The large number of fish sampled from Lake Alain and the absence of any brook trout mtDNA genotypes suggest that brook trout mtDNA has been eliminated from this population.

Discussion

Interspecific mtDNA exchanges have been reported in many animal groups including invertebrates (e.g., Powell 1983; Solignac and Monnerot 1986; Harrison et al. 1987; Aubert and Solignac 1990), amphibians (e.g., Spolsky and Uzzell 1984; Lamb and Avise 1986), reptiles (e.g., Wright et al. 1983), and mammals (e.g., Tegelstrom 1987; Lehman et al. 1991). In fish, interspecific transfer of mtDNA has been documented for several species (reviewed in Billington and Hebert 1991). However, definite cases of introgression are rare and have generally been observed at very low frequencies (Billington et al. 1988; Bernatchez et al. 1989; Avise et al. 1990; Bernatchez and Dodson 1991; Wilson and Hebert 1993). To our knowledge, complete interspecific replacement of the mitochondrial genome in natural populations has been described in only one fish species complex, *Notropis cornutus/chrysocephalus* (Dowling et al. 1989; Dowling and Hoeh 1991). Therefore,

the fixation of mitochondrial genome in Lake Alain brook trout is unusual and so far unique in salmonids.

Alternative hypotheses to introgression might explain the distinct mtDNA composition of this population. First, ancestral polymorphic mtDNA may have been retained in different taxa since they diverged from a common ancestor. Second, identity with the Arctic char mitochondrial genome may have resulted from convergent evolution. Generally speaking, it is often very difficult to distinguish which factor (or combination of factors) is responsible for the sharing of genetic variants in different taxa (discussed in Verspoor and Hammar 1991). However, alternative hypotheses to introgression can easily be ruled out in the present case. mtDNA variation observed in all available studies (Grewe et al. 1990; Danzmann et al. 1991*a*, 1991*b*; Ferguson et al. 1991; Bernatchez and Danzmann 1993; Wilson and Hebert 1993; C. Wilson, unpublished results) dealing with *S. alpinus* and *S. fontinalis* showed that no mtDNA genotypes are shared by both species over their range of distribution, which is inconsistent with a symplesiomorphic scenario. Secondly, net mtDNA sequence divergence between genotypes of both species is relatively high, estimated at 3.0% by Grewe et al. (1990).

This implies that even the most parsimonious scenario would require a high number of homoplastic events to explain a confusion of mtDNA variants in brook trout from Lake Alain with those of Arctic char, making the hypothesis of convergence very improbable. Therefore, there is no doubt that the mtDNA genotype observed in all brook trout from Lake Alain has been incorporated through introgression with Arctic char. Because Arctic char has never been found in any lake of the Portneuf River system despite intensive sport fishing, and biological surveys conducted over the past 30 years, it is most likely that these introgressive events are ancient and that their effect has persisted for a long time. Therefore, the present case clearly illustrates that introgressive hybridization may have a permanent effect on the genetic makeup of animal species. We are currently extending our survey to verify if introgressed populations of brook trout are also present in other river systems.

The fact that fish from Lake Alain were otherwise indistinguishable from brook trout based on morphological and allozyme criteria substantiates that nuclear introgression has not occurred or has long been diluted. Natural hybridization between sympatric Arctic char and brook trout populations in Labrador has revealed both morphological and nuclear intermediacy in the hybrids, supporting the latter scenario. It is possible that the present situation observed in char and trout from Lake Alain arose by repeated backcrossing of female hybrids with male brook trout until the Arctic char nuclear genome eventually disappeared. The lack of nuclear introgression in brook trout from Lake Alain further corroborates the hypothesis that complete interspecific replacement of the mitochondrial genome is an ancient event.

The evolutionary impact that the permanent mitochondrial replacement may potentially have on that population is directly related to the neutral or selective nature of variation between the mitochondrial genome of brook trout and that of Arctic char. Most if not all intraspecific variation detected in the mitochondrial genome is generally accepted to be neutral. This concept of mitochondrial neutrality has been extended to explain interspecific transfer of mtDNA where introgression has occurred (e.g., Tegelstrom 1987; Dowling and Hoeh 1991). It has also been reinforced by theoretical work demonstrating that mitochondrial gene flow across species boundaries is likely, unless the fitness of resulting offspring is very low (Takahata and Slatkin 1984). However, the acceptance of neutrality without further consideration of adaptibility has hampered our understanding of the relative roles of deterministic and stochastic processes as driving forces of gene exchange among different taxa (Arnold 1992). Indeed, a strict test of neutralist versus selectionist hypotheses will require an experimental approach that can substantiate predictions of differential response between introgressed and pure populations (Powers et al. 1991).

The permanent replacement of brook trout mtDNA with that of Arctic char could be selectively significant. Differences in the two mitochondrial genomes may be manifested physiologically, since several mitochondrial enzymes, central to intermediary metabolism, are partly encoded by mitochondrial genes. For example, metabolic thermosensitivity in ectotherms has been directly linked to the thermosensitivity of a mitochondrial enzyme, cytochrome oxydase (Blier and Guderley 1994). The activity of this enzyme, therefore, may reflect the organism's capacity towards thermal adaptation. Genetic differences between the mitochondrial genomes of introgressed and nonintrogressed populations could conceivably translate to differences in the metabolic capacity of the different mitochondria and, hence, their adaptiveness to environmental temperature. Indeed, the Arctic char is typically more adapted to cold environments than is brook trout, as exemplified by its more northern distribution and lower thermal optimum (Beamish 1980; Johnson 1980). In such a case, we can hypothesize that under low temperature constraints, there is a selective advantage imparted towards introgressed brook trout in having the mitochondrial genome of Arctic char. We are currently testing this prediction by experimentally assessing differences in thermosensitivity between introgressed and pure brook trout individuals at the biochemical, cellular, and organism levels of organization.

Clearly, experimentation is needed to determine if the fixation of Arctic char mtDNA in this population has resulted from deterministic (selection) or stochastic (historical) events. Together, experimentation and observation at several levels could potentially provide new insights into selection of both nuclear and mitochondrial gene flow.

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Allometric and environmental correlates of territory size in juvenile Atlantic salmon (*Salmo salar*)[1]

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Abstract: We observed juvenile Atlantic salmon (*Salmo salar*) over a wide range of stream conditions and body sizes to determine the relative importance of environmental factors and body size as predictors of territory size. Defended and foraging areas were virtually identical in size. Eighty-eight percent of the variation in territory size was explained by differences in body size and age; territory size increased with body size, but young-of-the-year salmon had relatively larger territories for their body size than older fish. Territory size was inversely related to food abundance, the only significant environmental correlate of territory size, but food only explained an additional 2% of the variation in territory size. Intruder pressure, visual isolation, and current velocity were not significantly related to territory size. The allometric scaling of territory size was consistent with the hypothesis that Atlantic salmon maintain a maximum daily ration of drift flowing over their territory. Atlantic salmon in Catamaran Brook have larger territories than is reported in the literature for other stream-dwelling salmonids of a similar size.

Résumé : Nous avons observé des juvéniles de saumon atlantique (*Salmo salar*) dans une vaste gamme de conditions lotiques et de tailles corporelles pour mesurer l'importance relative des facteurs environnementaux et de la taille corporelle comme prédicteurs de la taille du territoire. Les zones de défense et d'alimentation étaient pratiquement de taille identique. On pouvait expliquer 88 % de la variation de la taille du territoire par des différences dans la taille corporelle et dans l'âge; la taille du territoire augmentait avec la taille corporelle, mais les jeunes de l'année occupaient des territoires relativement plus grands pour leur taille corporelle que les poissons plus âgés. La taille du territoire était inversement reliée à l'abondance de nourriture, seul corrélat environnemental important, mais l'alimentation expliquait seulement une part supplémentaire de 2 % de la variation de la taille du territoire. La pression des intrus, l'isolement visuel et la vitesse du courant n'étaient pas directement liés à la taille du territoire. L'échelle allométrique de la taille du territoire concordait avec l'hypothèse selon laquelle les saumons atlantiques maintiennent une ration quotidienne maximale de matières nutritives en dérive au-dessus de leur territoire. Les saumons atlantiques du ruisseau Catamaran occupent des territoires plus grands que ne le signale la littérature pour d'autres salmonidés lotiques de taille similaire.

[Traduit par la Rédaction]

Introduction

Ecologists have approached the study of territory size in two fundamentally different ways. Allometric studies typically relate the average territory size of a species to its body mass and diet. In general, these interspecific studies have shown that territory size (or home range) increases directly with body mass and inversely with the amount of plant matter in the diet (McNab 1963; Schoener 1968; Harestad and Bunnell 1979). Alternatively, an optimality approach (e.g., Schoener 1983) predicts how an individual should change its territory size in response to changing environmental conditions. These studies have shown that territory size is usually inversely related to intruder pressure (e.g., Myers et al. 1979; Norman and Jones 1984) or food abundance (e.g., Stimson 1973; Simon 1975; Hixon 1981). Optimal territory size models are usually tested intraspecifically, but typically ignore the variation in body size found within wild populations (e.g., Dill et al. 1981). In contrast, allometric studies have rarely been applied intraspecifically, perhaps because of a lack of variation in body size.

Both an allometric and optimality approach have been applied to the study of territory size in stream-dwelling salmonid fish. Optimal territory size models predict that territory size will be influenced by food abundance and intruder

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pressure (Dill 1978; Grant and Noakes 1987). Tests of these models suggest that territory size decreases with increasing food abundance (Dill et al. 1981), intruder pressure (Dill et al. 1981; McNicol and Noakes 1984), or a combination of both (Slaney and Northcote 1974). Allometric studies show that body size explains between 69 and 97% of the variation in territory size (Grant et al. 1989; Elliott 1990; Grant and Kramer 1990), apparently leaving little residual variation to be explained by environmental variables.

Salmonid biologists have been interested in territory size because it is thought to limit population density (Chapman 1966; Allen 1969; Gibson 1993). If territory size is influenced by both environmental conditions and body size, then both an allometric and an optimality approach will be necessary to predict territory size and, ultimately, maximum population density (Grant and Kramer 1990). Hence, the purpose of our study was to develop a predictive model of territory size for Atlantic salmon (*Salmo salar*) using both approaches.

We conducted a field study of juvenile Atlantic salmon to determine the relative importance of body size, food abundance, and intruder pressure as predictors of territory size. We tested the predictions that territory size will increase with increasing body size and decrease with increasing food abundance or intruder pressure. In addition, we examined other ecological variables that could potentially influence the costs or benefits of defence for stream-dwelling salmonids. In Atlantic salmon, for example, increasing substrate size apparently produces greater visual isolation for a territory holder and reduces territory size (Kalleberg 1958), perhaps because the costs of expelling intruders increase (Eason and Stamps 1992). Optimal territory size models would also predict a decrease in territory size with increasing current velocity because of an increase in the costs of swimming and the costs of defence (e.g., Cole and Noakes 1980). We combined measurements of each of these variables in a multiple regression model to determine their importance in predicting the territory size of juvenile Atlantic salmon.

Materials and methods

Study site and species

Catamaran Brook, a third-order tributary of the Little Southwest Miramichi River (46°52.7′N, 66°06.0′W), is a productive salmon stream in central New Brunswick (Cunjak et al. 1990). Observations of juvenile salmon were made in the lower 2 km of the brook and in a 1-km section, 5 km upstream from the mouth of the brook. A naturally reproducing population of anadromous Atlantic salmon spawn in the stream from mid-October to mid-November each fall. Young-of-the-year salmon emerge from gravel nests by mid-June at about 2.6 cm in length (Randall 1982) and remain in the stream until they are 2–3 years of age or about 12 cm in length (Cunjak et al. 1993). Atlantic salmon are the most abundant fish species in the stream. A more detailed description of the stream and its biota can be found in Cunjak et al. (1990, 1993).

Measurement of territory size

We measured the territories of 48 fish (hereafter the focal fish) from 15 June to 1 September 1992, between 09:00 and 16:00, by observing individual fish defending areas along the stream bottom. For 25 individuals, we recorded aggressive interactions from behind a portable blind using a video camera mounted on a tripod. Aggressive distance was defined as the distance between the focal fish and the intruder when the latter elicited an aggressive response. We attempted to record at least 10 interactions with intruders to describe the size of the territory. After the observation period, we videotaped a metre stick at many different angles on the fish's territory to permit measurement of the actual aggressive distances from the videotaped image.

The video camera could not be used for another 23 individuals in faster current because of surface disturbance. In these habitats, we observed fish by snorkelling. Although the fish were sensitive to overhead movement, approaching from underwater and downstream did not appear to disturb them. By noting the position on the stream bottom where intruding fish first elicited an aggressive response by the focal fish, we constructed a territory map. The observer recorded the aggressive interactions with intruders by sketching a territory map on an underwater slate. Actual measurements of the aggressive distances were made after the observation period.

Because there were fewer than 10 aggressive interactions for 20 of 48 focal fish by the end of the observation period (mean = 90 minutes, range = 75–120), we created interactions by carefully disturbing a neighbouring fish, causing it to swim across the territory boundary of the focal fish. To disturb a fish, the observer moved a 1.5-m branch, underwater, towards the fish. When the tip of the branch was within a few centimetres of the fish, it normally swam away from the branch, often across the territory of the focal fish and was chased. This procedure did not seem to disturb the focal fish because it appeared to interact normally and continue feeding.

From the videotapes, we also measured foraging distance, the distance from a fish's holding station to the point where it intercepted a potential food item. Although fish did not always eat the intercepted item, all attempts were measured.

After the observation period, we captured the focal fish in 46 of 48 cases. The body size of the two missing fish was estimated by comparing their lengths with objects of known size from the videotapes. To be certain that we captured the fish we were observing, each focal fish had to remain in the view of the video camera or snorkeller for the entire observation period and until it was captured. If there was any uncertainty about the fish's identity, we terminated the observation. Captured fish were weighed (±0.05 g), measured for fork length to the nearest 0.5 mm, and preserved in 10% buffered formalin for later analysis of stomach contents. Scale samples were taken for aging.

Measurement of environmental variables

After capturing or attempting to capture the focal fish, we measured several environmental variables that may affect territory size. To estimate food abundance, we sampled drift flowing through the fish's territory. Shortly after observing a fish (11:00–18:00), we took a 1-h drift sample, centred on the territory, using a drift net 1 m long with an opening of 15.2 × 23 cm and a mesh size of 300 μm.

The mouth of the drift net was constructed with a rectangular metal collar that was 12.5 cm deep, to maintain a laminar flow at the mouth of the net and to prevent backwashing (see Field-Dodgson 1985). In shallow habitats, where the drift net was not completely submerged, we adjusted our estimate of food abundance on a territory so that the unit of area sampled was standardized. Each drift sample was placed in a holding jar and sorted, usually that evening or the next morning. Inedible material, such as decomposing leaves and insect exuviae, was removed from the sample and the remaining animals were stored in 10% buffered formalin. Preserved animals were later measured using a microscope fitted with an ocular micrometer. In order to eliminate prey items which were either too small or too large to be eaten by a focal fish, we used the stomach contents of the 46 captured fish to predict the maximum and minimum prey size for each focal fish. Average, maximum, and minimum prey sizes ingested were positively related to the fork length of the fish (Keeley 1993). To estimate maximum and minimum potential prey available in the drift samples, we used the upper 95% prediction interval from maximum-prey-size regression and the lower 95% prediction interval from the minimum-prey-size regression, respectively. In most cases, however, we did not have to eliminate any of the prey items in the drift sample. Once drift samples were sorted into edible prey items, each sample was dried at 50°C for 24 h and then moved to a desiccator for an additional 24 h. Each sample was then weighed at 24-h intervals until a stable weight (±0.0002 g) was obtained (McCauley 1984). We used dry weight of food items per square centimetre per 1-h drift sample as our estimate of food density on a territory.

To estimate intrusion pressure on a territory, we counted the number of aggressive interactions with intruders on the territory per hour. As a second measure of intruder pressure, we estimated local density by counting the number of fish in a 1- and 2-m^2 area surrounding the focal fish every 15 minutes during an observation period. This method of estimating density will be higher than a random sample of fish density because we centred the area sampled around the focal fish to determine the density of fish that each focal fish experienced.

We used a Marsh–McBirney current meter to measure current velocity at the holding station, the location where the fish normally held its snout. We also recorded current velocity higher in the water column where the fish fed and at secondary holding stations.

We quantified visibility on the territory in two ways. As a measure of rugosity, we traced the profile of the stream bottom parallel and perpendicular to the current in 20-cm intervals using a stiff, flexible cord. The distance followed by the cord was then divided by the linear distance to give a mean rugosity for the territory. Second, we quantified substrate type by placing one or two meter sticks parallel and perpendicular to the current. We then determined the dominant substrate type at 10-cm intervals as being (1) <2 mm (sand, silt), (2) 2–16 mm (gravel), (3) 17–64 mm (pebble), (4) 65–256 mm (cobble), or (5) >256 mm (boulder) (after Bain et al. 1985). The 10-cm intervals were then averaged to give a mean substrate type for the territory.

We also recorded water temperature (±0.2°C) at the beginning and end of an observation period and depth to the nearest 0.5 cm at the centre of the territory.

Statistical analyses

We used two different methods to calculate the area defended by the fish. The maximum convex polygon method (sensu Schoener 1981) uses the outermost points where the territory holder expelled intruders. The disadvantage of this method is that it is extremely sensitive to sample size. To correct for this problem when we had more than 10 estimates of the territory radius, we used a BASIC computer program to randomly select 10 points from the available number and calculate the maximum convex polygon from these data. This procedure was repeated 1000 times for each territory and the mean of these values was used as an estimate of territory size. We also used the mean aggressive distance for a fish to estimate territory radius and then calculated territory area as the area of a circle. Although salmonid territories may be eccentric (Noakes and McNicol 1982), stream-dwelling salmonids typically feed and defend a territory from a central place. Therefore, a circle is a reasonable approximation of territory shape (Grant et al. 1989). The advantage of this "circular" method is that it will not be biased by sample size.

To determine which method of calculating territory size was the most appropriate, we compared the maximum convex polygon and circular territory area with the area used by the fish while foraging. Models of optimal territory size assume that the area used by an animal when feeding should be equal to the area defended (Schoener 1983). Because foraging attempts were much more frequent than aggressive acts, we randomly selected 100 foraging attempts from each of the videotaped territories ($n = 25$) to calculate the foraging area using the maximum convex polygon method.

We used a multiple regression approach to determine if any of the measured variables could account for variation in territory size. Unless otherwise stated, we used an ordinary least squares (OLS) regression. All multiple regression models were analyzed for multicollinearity to ensure that the assumptions of the OLS technique were met (Neter et al. 1990). For simple regressions, when both independent and dependent variables had similar ranges in error variance, we used a reduced major axis (RMA) regression as an alternative to the OLS (McArdle 1988; LaBarbera 1989). Each of the variables was tested for normality and, when required, was $\log_{10}$ transformed.

Results

Aggressive behaviour

Juvenile Atlantic salmon defend territories over a wide range of stream conditions (Table 1). Much of their time is spent on or near the stream bottom, facing into the current. As observed in previous studies (e.g., Kalleberg 1958; Keenleyside 1962; Arnold et al. 1991), juvenile Atlantic salmon appear to use their pectoral fins as hydrofoils to generate negative lift against the stream bottom.

Fish typically held position at one centrally located spot, their primary station, but used other stations to varying degrees. On average, 77% of aggressive acts originated

Table 1. Summary of territory characteristics for 48 juvenile Atlantic salmon.

Variable	Mean	Range	CV[a]
Territory size (m^2)	0.55	0.02–7.46	205
Body size (fork length, cm)	6.38	2.90–14.50	50
Abundance of drift (mg dry wt·cm^{-2}·h^{-1})	141.4	10.5–518.7	80
Intruder pressure (intrusions·h^{-1})	8.13	1–21	58
Current velocity (cm·s^{-1})	21.0	1–45	54
Rugosity[b]	1.1	1.0–1.3	6
Temperature (°C)	16.5	12.7–21.0	10
Fish density (no.·m^{-2})	3.2	1.0–6.1	40
Depth (cm)	31.5	4.0–67.3	47
Substrate type (mean)[c]	3.1	2.0–4.1	13

[a]Coefficient of variation (SD/mean × 100).
[b]Contour length of transect/linear distance of transect.
[c]Categorized according to Bain et al. (1985).

from their primary station. Upon encountering an intruding fish, the focal fish would dart out from its station, expel the intruder, and return. Twelve percent of aggressive interactions were from a secondary station and 6% were from a tertiary station.

The frequency of aggressive behaviour by the 48 focal fish was low (mean = 8.1·h^{-1}, range = 1–21·h^{-1}). The low rate of aggression was primarily because of a low rate of intrusion on the territories; intruders were expelled by focal fish virtually 100% of the time. The intruders were composed of 96% Atlantic salmon, 3.6% blacknose dace (*Rhinichthys atratulus*), and 0.8% brook trout (*Salvelinus fontinalis*). Although 13 other species of fish occur in Catamaran Brook (Cunjak et al. 1993), they were not observed intruding on the territories of focal fish.

Of the 719 aggressive acts that we observed, 94% were charges, a rapid approach by the focal fish towards an intruder. If the intruder did not quickly flee, it was struck by the focal fish's mouth. The remaining 6% of aggressive interactions were lateral displays. A lateral display occurred when a focal fish erected its paired and median fins maximally and moved laterally toward an intruder. Ninety percent of the aggressive responses by focal fish were charges towards conspecifics.

Charges and lateral displays did not differ significantly in aggressive distance between the 24 fish that exhibited both behaviour patterns (paired $t = 0.85$, $n = 24$, $P = 0.41$). Similarly, the aggressive distance did not differ significantly between heterospecific and conspecific intruders (paired $t = 0.49$, $n = 13$, $P = 0.63$) or between natural and artificial interactions (paired $t = 0.65$, $n = 31$, $P = 0.52$). Therefore, we combined all aggressive interactions when calculating territory size, regardless of the species being expelled, the type of interaction (artificial or natural), or the behaviour exhibited by the focal fish.

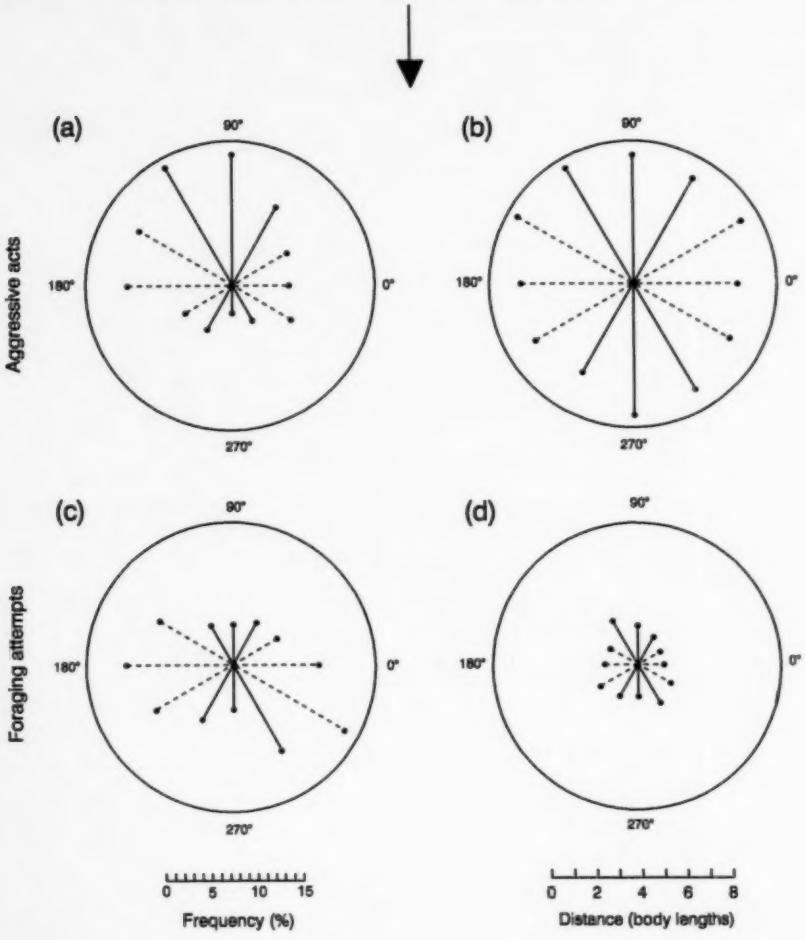
The defence effort by the focal fish was not equally distributed in all directions (Fig. 1*a*, Rayleigh's test, $P < 0.001$; Batchelet 1981). To compare the number and distance of aggressive acts between quadrants, we paired the analysis by individual. All comparisons have Bonneferoni-corrected α-values with P-critical = 0.008 (Neter et al. 1990). More aggressive acts were directed upstream compared with the downstream quadrant (paired $t = 3.38$, $n = 33$, $P = 0.002$) but not compared with the average of the two lateral quadrants (paired $t = 2.19$, $n = 45$, $P = 0.034$). Aggressive distance was greater upstream than laterally (Fig. 1*b*, paired $t = 3.69$, $n = 42$, $P = 0.001$) but not greater upstream than downstream ($t = 2.065$, $n = 32$, $P = 0.047$). Aggressive distances did not differ significantly between the average of the two lateral quadrants and the downstream quadrant (paired $t = 0.35$, $n = 29$, $P = 0.73$). Territories appeared to be bilaterally symmetrical because there were no differences between left and right lateral quadrants in either frequency of acts (Fig. 1*a*, paired $t = 1.15$, $n = 43$, $P = 0.16$) or aggressive distance (Fig. 1*b*, paired $t = 0.16$, $n = 43$, $P = 0.87$).

Foraging behaviour

Juvenile Atlantic salmon initiated most foraging attempts from their primary station. On average, 72, 14, and 6% of foraging attempts occurred from the primary, secondary, and tertiary stations, respectively. The remaining 8% of foraging attempts were initiated from as many as 30 different locations on a territory. However, many of these occurred while a fish was returning to its original station from a previous foraging attempt. Foraging attempts were much more frequent than aggressive interactions (mean = 316.4·h^{-1}, range = 106–540·h^{-1}). Most foraging attempts were directed at potential prey in the water column; less than 1% were directed at the surface of the stream. Benthic foraging appeared to be even less frequent than surface foraging, but was often difficult to distinguish on the videotapes.

The salmon foraged in all directions (Fig. 1*c*), but their effort was not distributed equally (Rayleigh's test, $P < 0.001$). There were more foraging attempts on average in the lateral quadrants than upstream (Fig. 1*c*, paired $t = 7.15$, $n = 25$, $P < 0.001$) but not downstream ($t = 2.18$, $n = 25$, $P = 0.035$). The number of foraging attempts did not differ significantly between the upstream and downstream quadrants (Fig. 1*c*, paired $t = 2.18$, $n = 25$, $P = 0.040$). Foraging distances did not differ between the lateral quadrants and upstream (Fig. 1*d*, paired $t = 1.61$, $n = 25$, $P = 0.12$) or downstream (paired $t = 2.11$, $n = 25$, $P = 0.046$).

Fig. 1. (*a*) Relative frequency and (*b*) average distance of aggressive interactions with intruders in 12 directions (719 acts by 48 focal fish). Dashed lines represent the lateral quadrants whereas solid lines facing the top and bottom of the circle represent the upstream and downstream quadrants, respectively. (*c*) Relative frequency and (*d*) average distance of foraging attempts in 12 directions (2500 attempts by 25 focal fish). For graphical purposes, all foraging attempts and aggressive acts are assumed to be initiated from a single station. The arrow indicates direction of the current.



Foraging distances were also not different between downstream and upstream quadrants (paired $t = 0.23$, $n = 25$, $P = 0.82$). Foraging areas appeared to be bilaterally symmetrical; there were no significant differences between left and right lateral quadrants in either frequency of foraging (paired $t = 0.36$, $n = 25$, $P = 0.72$) or foraging distances (paired $t = 0.91$, $n = 25$, $P = 0.37$).

Comparison of defended and foraging areas

Although both the areas defended and used for foraging were centred about the primary station, there were clear differences in the relative space use around this station (Fig. 1*b* versus 1*d*). The frequency of foraging decreased with distance from the primary station whereas the frequency of aggressive interactions was maximal at intermediate distances (Fig. 2). The mean aggressive distance was greater than the mean foraging distance in the lateral (paired $t = 12.32$, $n = 25$, $P < 0.001$), upstream (paired $t = 8.89$, $n = 25$, $P < 0.001$), and downstream (paired $t = 8.66$, $n = 21$, $P < 0.001$) directions (Figs. 1*c* and 1*d*). However, maximum lateral foraging radii did not differ significantly from maximum lateral aggressive radii (paired $t = 1.18$, $n = 25$, $P = 0.25$). These results suggest that there were relative but not absolute differences between the use of the defended and foraging areas. Furthermore, fish with larger aggressive radii had larger foraging radii that scaled to each other with a slope of 1.01 (Fig. 3). Even after we controlled for the effect of body size by including fork

Fig. 2. Frequency distribution of foraging attempts (open bars, $n = 2500$) and aggressive acts (solid bars, $n = 719$) in relation to the distance from a station.

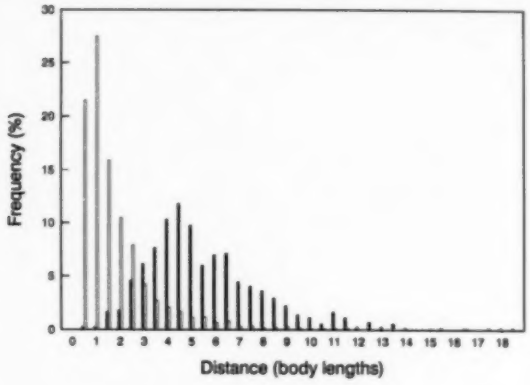
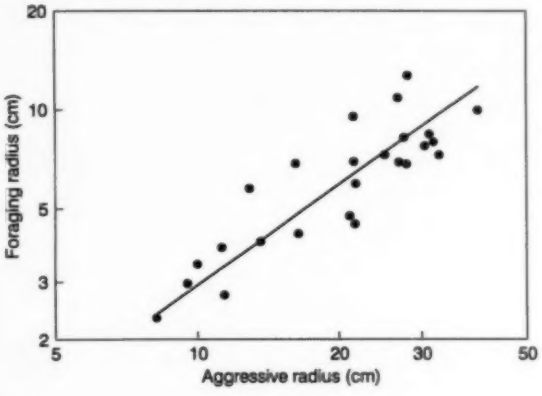


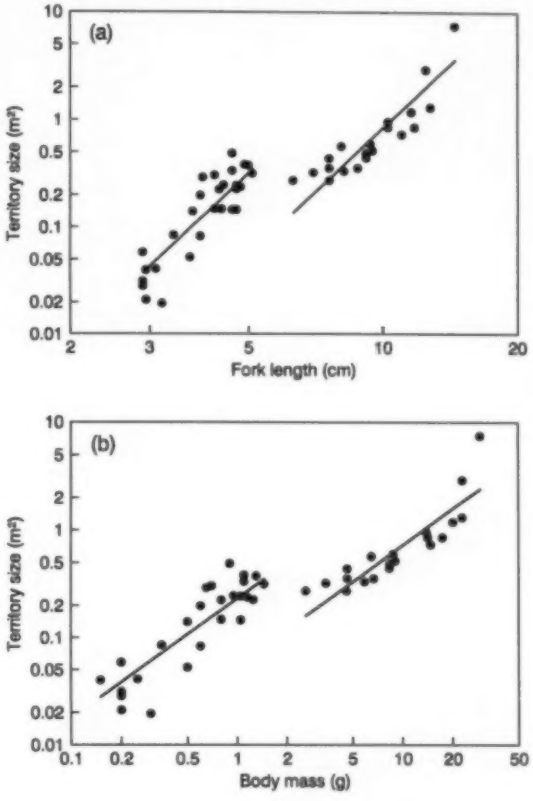
Fig. 3. Relationship between mean foraging radius and mean aggressive radius for 25 Atlantic salmon (reduced major axis regression: $\log_{10} Y = 1.01 \log_{10} X - 0.54$; $r^2 = 0.75$, $P < 0.001$).



length and aggressive radius in a multiple correlation, fish with larger aggressive radii had larger foraging radii (partial $r = 0.50$, $n = 25$, $P = 0.012$).

Because aggressive acts were so infrequent, we used the foraging data to test the accuracy of the two methods of calculating territory size (see Materials and methods). Both estimates of territory area were highly correlated with each other ($r = 0.91$, $n = 48$, $P < 0.001$), and although the circular method was significantly larger (paired $t = 3.62$, $n = 48$, $P = 0.001$), the mean difference was only 3.2%. In addition, both estimates of territory size were highly correlated with the foraging area (circular method: $r = 0.86$, $n = 25$, $P < 0.001$; convex polygon method: $r = 0.88$, $n = 25$, $P < 0.001$) and did not differ significantly from the foraging area (circular method: paired $t = 0.10$, $n = 25$, $P = 0.92$; convex polygon method: paired $t = 0.14$, $n = 25$, $P = 0.98$). However, the difference between the foraging area and the area calculated by the circular method (mean difference = 0.005 m^2) was slightly smaller than

Fig. 4. Relationship between territory size and (*a*) fork length for 48 juvenile Atlantic salmon ($\log_{10}$ territory size (m^2) = $-3.23 + 3.91 \log_{10}$ fork length (cm) $- 0.76$ age ($R^2 = 0.88$, $P < 0.0001$), where age is 0 for young-of-the-year or 1 for older fish), and (*b*) body mass for 46 juvenile Atlantic salmon ($\log_{10}$ territory size (m^2) = $-0.63 + 1.12 \log_{10}$ body mass (g) $- 0.63$ age ($R^2 = 0.86$, $P < 0.0001$). Fish less than or equal to 5.1 cm or 1.45 g are young-of-the-year; all others are older fish.

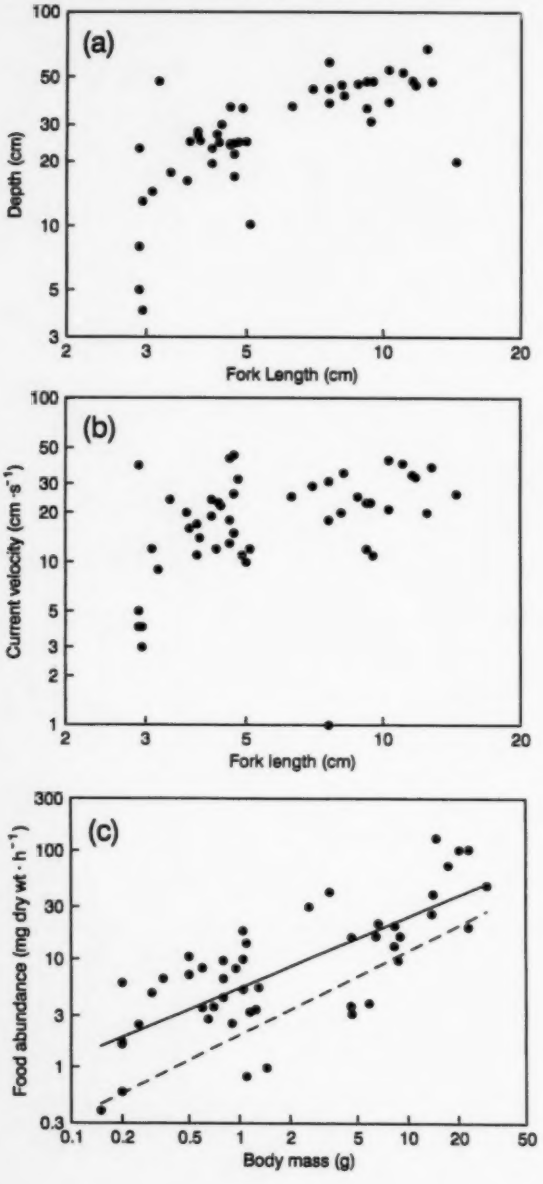


the difference between the foraging area and that calculated by the polygon method (mean difference = 0.01 m^2). Therefore, we used the circular method to estimate territory area in all subsequent analyses.

Allometry of territory size

Territory size increased with increasing body length (Fig. 4*a*). However, a change in the regression function occurred between young-of-the-year salmon and older individuals. The intercepts of the two lines differed significantly (ANCOVA: $F_{1,44} = 35.53$, $P < 0.0001$) but not the slopes (ANCOVA: $F_{1,44} = 3.79$, $P = 0.058$). Territory size increased with increasing body mass in a similar way (Fig. 4*b*); the intercepts of the lines differed significantly between the age categories (ANCOVA: $F_{1,42} = 22.72$, $P < 0.0001$) but not the slopes (ANCOVA: $F_{1,42} = 0.48$, $P = 0.49$).

Fig. 5. Relationship between fork length of 48 juvenile Atlantic salmon and (*a*) depth ($r = 0.70$, $n = 48$, $P < 0.001$) and (*b*) current velocity ($r = 0.43$, $n = 48$, $P = 0.002$) of their territories. (*c*) Relationship between the amount of food flowing through the territory of 46 juvenile Atlantic salmon per hour and body mass ($\log_{10} Y = 0.68 \log_{10} X + 0.72$; $r^2 = 0.57$, $P < 0.001$; solid line). The dashed line represents the maximum daily ration per hour predicted from Elliott's (1975*a*) model for brown trout.



The break in the allometric territory size line between young-of-the-year and older fish was unexpected. Older fish occupied deeper (Fig. 5*a*), faster (Fig. 5*b*) areas of the stream and hence may have required relatively smaller territories to acquire sufficient food. In fact, the amount of food flowing through a territory increased linearly with increasing body mass (Fig. 5*c*) and was significantly greater than the maximum ration predicted for brown trout (*Salmo trutta*, Elliott 1975*a*) of a similar size (38 of 46 points above the dashed line, Fig. 5*c*, binomial test: $P < 0.001$). Interestingly, the slopes of these two lines did not differ significantly from each other ($t = 1.01$, $P > 0.20$). Furthermore, if we assume that the young-of-the-year salmon emerged at a mass of 0.15 g on 13 June (cf. Randall 1982), then we can compare the growth rate of our fish with that of a similar-sized brown trout fed on maximum ration (Elliott 1975*b*, 1984) at the average temperature in Catamaran Brook (Cunjak et al. 1993). Young-of-the-year salmon grew at a rate not significantly different from the rate predicted for brown trout fed on a maximum ration (paired $t = 1.62$, $P = 0.12$).

Environmental correlates of territory size

We used a multiple regression analysis to evaluate which variables influenced territory size (Table 2). Besides body size and age, only food abundance was significantly related to territory size (Table 2). As predicted, territory size was inversely related to food abundance (Fig. 6). Separate analyses for each age group gave similar results to the overall analysis, with one exception. Territory size was significantly inversely related to food abundance only for young-of-the-year fish (Fig. 6).

Contrary to the predictions of optimal territory size models, territory size was not related to intruder pressure. This was true whether our estimate of intruder pressure was the number of aggressive interactions per hour (Table 2) or local population density (partial $r = 0.050$, $P = 0.75$). Similarly, territory size was not related to visibility whether we used the rugosity of the stream bottom (Table 2) or the average substrate size class (partial $r = 0.17$, $P = 0.28$). In addition, territory size was not related to current velocity (Table 2), water temperature (partial $r = -0.077$, $P = 0.63$), or depth (partial $r = -0.087$, $P = 0.59$). To ensure that these correlations were not caused by or obscured by the effect of season, we included day of the year in the regression model. Territory size was not related to the day on which we observed each of the 48 fish (partial $r = -0.19$, $P = 0.24$).

The multiple regression model that best describes territory size in juvenile Atlantic salmon is $\log_{10}$ territory size (m^2) $= -2.80 + 3.83 \log_{10}$ fork length (cm) $- 0.72$ age $- 0.19 \log_{10}$ food abundance (mg dry wt·cm^{-2}·h^{-1}) ($R^2 = 0.90$, $P < 0.0001$), where age is equal to 0 for young-of-the-year and 1 for older fish.

Discussion

Defended versus foraging areas

Models of optimal feeding territory size assume that the size of the defended area will be equal to the size of the area used for foraging (Schoener 1983). On average, foraging attempts were closer to a station than aggressive interactions (Fig. 2). However, the frequency of foraging was 40 times greater than aggressive behaviour, so there was no significant difference between the size of foraging and

Table 2. Multiple regression statistics for several independent variables predicting territory size in juvenile Atlantic salmon.

Variable	Coefficient	SS[a]	*P*-value[a]
Fork length (cm)	3.864	4.394	0.0001
Age (0^+ vs. older)	−0.753	1.089	0.0001
Food abundance (mg dry wt·cm^{-2}·h^{-1})	−0.167	0.140	0.037
Intruder pressure (intrusions·h^{-1})	0.127	0.049	0.210
Current velocity (cm·s^{-1})	−0.001	0.005	0.699
Rugosity[b]	1.753	0.064	0.154

[a]Sum of squares and probability for each variable when entered into the model after all other variables.

[b]Contour length of transect/linear distance of transect.

defended areas. Hence, when calculated for a similar duration, our data support the assumption that defended and foraging areas are a similar size. Our observation that foraging attempts are closer to the primary station than aggressive interactions is consistent with models for animals that defend and forage from a central place (Andersson 1978; Schoener 1983). Animals defend near the perimeter of their territory to prevent competitors from consuming resources on their territory. But, because travel costs increase with the distance from the prey, central place foragers that encounter prey at a distance should consume prey more often, closer to the centre of their foraging area than further away (Andersson 1978). Therefore, as predicted by foraging models (Andersson 1978; Schoener 1979), the centre of the foraging area was used more frequently than the perimeter (Fig. 2). Presumably, fish defend areas even rarely used for foraging to prevent conspecifics from consuming the infrequent and highly profitable prey items that drift by.

Defended and foraging areas are not always similar in other species. In fishes with all-purpose territories, the area defended against nest predators or mating rivals is smaller or larger than the foraging area, respectively (Kawanabe 1981; Kohda 1984). In addition, the area defended by threespot damselfish (*Pomacentrus planifrons*) increases with the diet overlap between the owner and the intruding species (Thresher 1976). However, the area defended against conspecifics is usually similar to the foraging area, as in our study.

Correlates of territory size

The variation in territory size of juvenile Atlantic salmon was primarily (i.e., 82%) explained by differences in body size, as in previous studies of territory size in salmonid fishes. Body size explained 87% of the variation in territory size in an interspecific study (Grant and Kramer 1990), 69% in brook trout (Grant et al. 1989), and over 90% in brown trout (Elliott 1990).

Food abundance was the only significant environmental correlate of territory size, but explained only 2% of the variation in territory size. While optimality models predict either an increase or decrease in territory size with an increase in food abundance (Schoener 1983), most studies have found that territory size decreases with increasing food abundance (e.g., Stimson 1973; Simon 1975; Hixon 1981). For stream-dwelling salmonids, models of territory size also make contradictory predictions about the effect of food abundance. For instance, Dill (1978) predicted that territory size will either decrease or not change with increasing food abundance whereas Grant and Noakes (1987) predicted either a decrease or an increase, depending on the types of constraints involved. The data available to evaluate these predictions are also equivocal. Slaney and Northcote (1974) found an inverse relationship between food abundance and territory size, but did not consider the effects of intruder density. Other studies either failed to detect an effect of food density on territory size (Symons 1971; McNicol and Noakes 1984) or found an inverse relationship with the abundance of benthic invertebrates, on which they rarely feed, and no significant relationship with the abundance of drifting invertebrates (Dill et al. 1981). Our study suggests that food abundance has a small inverse effect on the territory size of juvenile Atlantic salmon.

We found no support for the prediction that territory size decreases with increasing intruder pressure, despite having high power to detect such an effect ($\lambda = 111.6$, $\beta < 0.01$; Cohen 1988). In contrast with our results, intruder

Fig. 6. Residual variation from a territory size versus body size and age regression in relation to the abundance of drift on territory (one-tailed test: $r = -0.37$, $n = 48$, $P = 0.005$). Solid circles represent young-of-the-year fish (one-tailed test: $r = -0.56$, $n = 28$, $P = 0.001$) and open circles represent older fish (one-tailed test: $r = -0.10$, $n = 20$, $P = 0.339$).

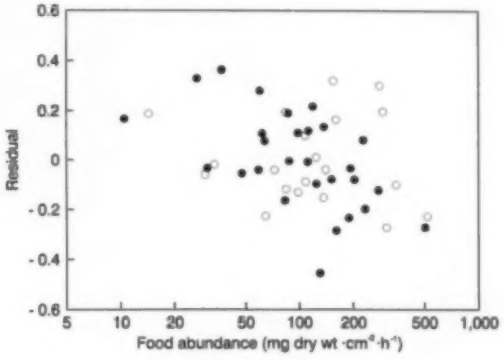
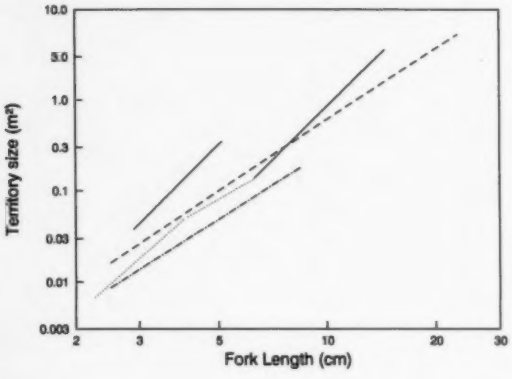


Table 3. Scaling of territory size to body length and mass in stream-dwelling salmonids.

Species	Length[a]	Mass[b]	Reference
Atlantic salmon	3.91 (3.28–4.55)	1.12 (0.91–1.33)	This study
Brook trout	2.48 (1.74–3.22)	0.82	Grant et al. 1989
Brown trout			
<4 cm	3.45 (3.40–3.51)	0.99	Elliott 1990
>4 cm	2.24 (2.17–2.31)	0.82	Elliott 1990
Interspecific	2.61 (2.15–3.07)	0.86	Grant and Kramer 1990

[a]Slope (95% CI) from territory size vs. body length regressions.
[b]Slope (95% CI) from territory size vs. body mass regressions. Coefficients for regressions from Grant et al. (1989) and Elliott (1990) were calculated from length–mass regressions given in Grant and Kramer (1990) and Elliott (1985), respectively.

Fig. 7. Regression lines of territory size versus fork length for brook trout (dashed–dotted line; Grant et al. 1989), brown trout (dotted line; Elliott 1990), an interspecific salmonid study (dashed line; Grant and Kramer 1990), and Atlantic salmon (solid lines; this study).



pressure is more important than food abundance in determining territory size in sanderlings (*Calidris alba*) (Myers et al. 1979) and coral reef fishes (Norman and Jones 1984; Tricas 1989). Both brook trout (McNicol and Noakes 1984) and coho salmon (*Oncorhynchus kistuch*) (Dill et al. 1981) defended smaller territories when intruder pressure or population density was higher. However, the densities of fish used by McNicol and Noakes (1984) were 4–10 times greater than the highest densities in Catamaran Brook, and the rate of intrusions observed by Dill et al. (1981) were 10 times greater than observed in Catamaran Brook. Given that Catamaran Brook is at least a moderately productive stream for Atlantic salmon (Cunjak et al. 1990), the level of intruder pressure required to exert a significant effect on territory size may rarely occur under natural conditions.

Current velocity had no significant effect on territory size. Cole and Noakes (1980) suggested that the costs of foraging in a stream increase with increasing current velocity. However, Atlantic salmon place their pectoral fins against the stream bottom to minimize the amount of energy required to hold position in the current (Arnold et al. 1991). As a result, they can maintain position at current speeds exceeding the maximum sustained swimming speeds of other salmonids or most other fishes of a similar size (Beamish 1978). Therefore, current velocity may have less effect on the defence costs for Atlantic salmon than for other stream-dwelling salmonids.

By increasing the degree of visual isolation, Kalleberg (1958) was able to reduce the territory size of Atlantic salmon. The natural variance in visual isolation in Catamaran Brook, measured by substrate rugosity and size, was low and had no significant effect on territory size. Most fish occupied areas where their field of vision was unobstructed.

Allometry of territory size

Atlantic salmon in Catamaran Brook have larger territories than other salmonids of a comparable size (Fig. 7). For example, 38 of 48 territories were larger than predicted by Grant and Kramer's (1990) interspecific line (binomial test: $P < 0.001$). The generality of our data is strengthened by similar measurements of territory size for Atlantic salmon in a Scottish stream (Stradmeyer and Thorpe 1987).

The scaling of territory size with body size was higher in our study than in other studies of salmonids. The slope of the territory size versus body length regression was significantly higher for Atlantic salmon in Catamaran Brook than for brook trout ($t = 4.54$, $P < 0.001$), an interspecific regression ($t = 4.13$, $P < 0.001$), and brown trout larger than 4 cm ($t = 5.30$, $P < 0.001$) but not those smaller than 4 cm ($t = 1.46$, $P > 0.1$) (Table 3). The scaling of territory size on body mass ranged from 1.12 for Atlantic salmon to 0.82 for brook trout (Table 3). If salmonid fish increase territory size to meet increasing energetic demands as they grow, then territory size and metabolic rate should scale in a similar way to body mass. In salmonids, metabolic rate scales to body mass by exponents that range from 0.78 to 0.97 (Brett 1965). Previous studies (Table 3) are all consistent with the metabolic rate hypothesis. Our slope was not significantly different from Brett's (1965) highest exponent ($t = 1.51$, $P > 0.1$) but was greater than 0.78 ($t = 3.37$, $P < 0.002$). Unlike previous researchers, we were able to measure the amount of food flowing across a territory (Fig. 5*c*). More than a maximum daily ration of food flowed through a territory in 12 h, but not all food that is available is detected and eaten (Wilzbach and

Cummins 1986). However, if we assume that a brown trout and an Atlantic salmon of a given size have the same energetic requirements, the similarity in the scaling of drift abundance (Fig. 5*c*, exponent = 0.68, 95% CI = 0.50–0.86) and maximum daily ration (exponent = 0.77; Elliott 1975*b*) suggests that Atlantic salmon increase their territory size to achieve a maximum ration. This hypothesis was supported by the observation that young-of-the-year salmon were growing at a rate not significantly different from a maximum rate predicted for brown trout (Elliott 1975*b*). Hence, the modest growth rate of Atlantic salmon in Catamaran Brook (Cunjak et al. 1993) appears to be due to a short growing season and cool water temperature rather than a shortage of food. However, this interpretation will depend on the applicability of Elliott's (1975*b*) brown trout model for Atlantic salmon.

A potential consequence of territoriality is the limitation of population density. If salmonids require more space as they grow, then population density will decrease with increasing body size. Stream-dwelling salmonids may, therefore, self-thin in a manner similar to plants because territories increase with body size (Grant and Kramer 1990; Elliott 1993; Grant 1993). Our data suggest that Atlantic salmon in Catamaran Brook have larger territories that scale more rapidly with body length than other stream salmonids. If this is a general result for Atlantic salmon, then the maximum densities of Atlantic salmon may be lower than those for other stream-dwelling salmonids. The validity of this prediction remains to be tested.

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Regional precipitation and surface water chemistry trends in southeastern Canada (1983–1991)

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Abstract: We analyzed for trends of acidification related variables from 1983 to 1992 in precipitation concentrations and deposition at six sites and surface water concentrations at 111 sites located from central Ontario to eastern Newfoundland. Precipitation showed significantly decreasing H^+ and SO_4^{2-} concentrations and deposition in central and eastern Ontario and at one of two sites in Québec (deposition only). For Ontario surface water sites, only increasing or stable SO_4^{2-} trends were observed, and these had both concomitant increasing and decreasing trends for pH and (or) acid-neutralizing capacity (ANC). Despite a considerable number of lakes showing decreasing SO_4^{2-} trends in Québec, pH and ANC also decreased. Québec was also the only region showing extensive evidence of increasing NO_3^-. The opposite situation was observed in Atlantic Canada lakes where despite increasing SO_4^{2-}, the dominant trend observed for pH and ANC was increasing. Trends observed for pH and ANC were used to classify the acidification status of our surface water sites: 60 of the 111 sites were found to be stable (i.e., showing no trends), 17 were continuing to acidify, and 34 were improving.

Résumé : Nous avons analysé les tendances temporelles monotones de variables chimiques reliés à l'acidification pour les concentrations et les dépôts des précipitations à six sites et pour les concentrations chimiques des eaux de surface à 111 sites répartis du centre de l'Ontario jusqu'à l'est de Terre-Neuve. Les précipitations ont montré des diminutions significatives des concentrations et des dépôts de H^+ dans le centre et dans l'est de l'Ontario et à un des deux sites du Québec (seulement les dépôts). Pour les sites d'eau de surface de l'Ontario uniquement, l'absence de tendances ou des augmentations de concentrations de SO_4^{2-} ont été observées et, dans deux cas, étaient accompagnées d'augmentations ou de diminutions concomitantes du pH et (ou) de la capacité neutralisant d'acide (CNA). En dépit du nombre considérable de lacs du Québec montrant des diminutions des concentrations de SO_4^{2-}, le pH et la CNA diminuaient aussi. Le Québec était également la seule région montrant une évidence considérable d'augmentations des concentrations de NO_3^-. La situation est inversée pour les lacs des provinces atlantiques où, en dépit des hausses de SO_4^{2-}, la tendance dominante observe pour le pH et la CNA était une augmentation. Les tendances observées pour le pH et la CNA ont été utilisées pour classer l'état d'acidification de nos sites d'eau de surface : 60 des 111 lacs étaient stables (i.e., ne montrant aucun tendance), 17 ont continué de s'acidifier et 34 se sont améliorés.

[Traduit par la Rédaction]

Introduction

East of the Ontario–Manitoba border and south of 52°N latitude, Canada has more than 700 000 lakes considered sensitive to acidification based on geological characteristics and atmospheric sulphate (SO_4^{2-}) deposition (Jeffries 1991; Hélie et al. 1993). In the early 1980s, a number of monitoring programs commenced (e.g., Bouchard and Haemmerli 1992; Clair et al. 1992) to provide information for assessing

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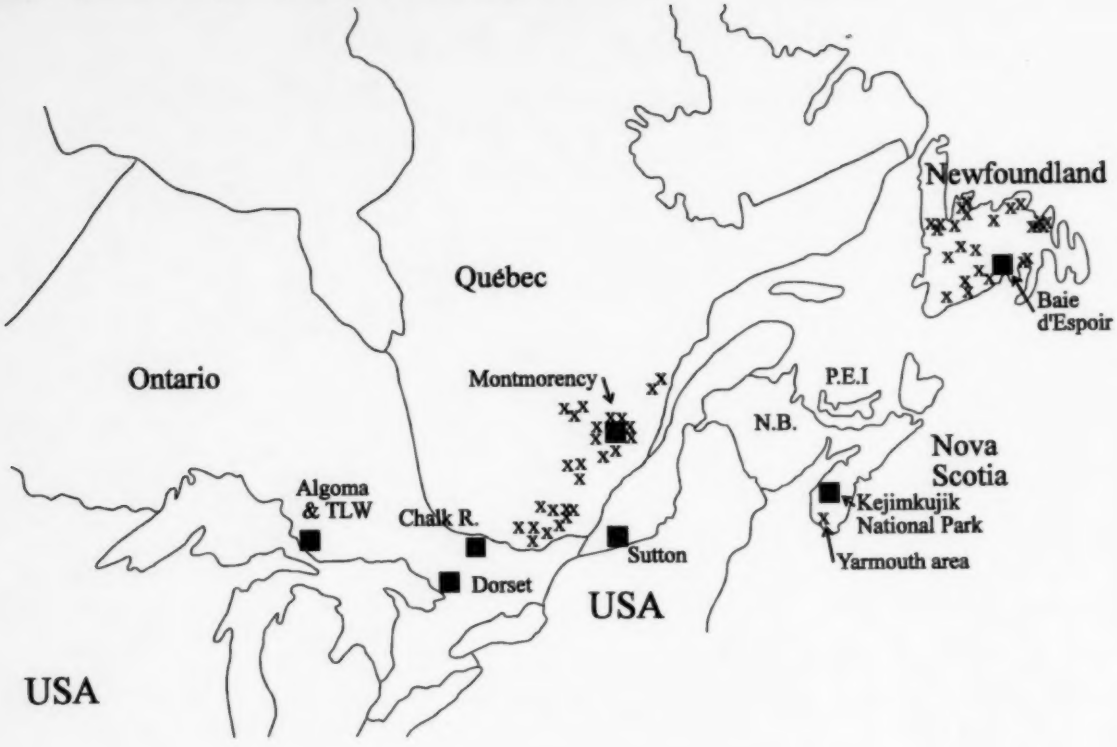
Fig. 1. Location of study sites. Precipitation sites are named.



the effect of acid deposition and in the change of the acid deposition rate on surface water quality. Intensive watershed monitoring was established at several sites also, e.g., the "calibrated watersheds" at Turkey Lakes in central Ontario (Jeffries et al. 1988), Harp and Plastic lakes in south-central Ontario (Dillon and LaZerte 1992), Lac Laflamme in southern Québec (Papineau 1987), and Kejimkujik watersheds in Nova Scotia (Kerekes and Freedman 1989).

An important objective of the aquatic monitoring programs is to document changes in water chemistry resulting from changes in SO_2 emissions. For example, in the Sudbury region of Ontario, Dillon et al. (1986) and Keller et al. (1992) reported decreases in lake SO_4^{2-} and increased pH and acid-neutralizing capacity (ANC) following reductions in local SO_2 emissions in the late 1970s. A similar pattern was reported by Kelso et al. (1992) in the Algoma region of central Ontario, although it was unrelated to the changes at Sudbury. In Nova Scotia, Sirois and Summers (1989) observed a downward trend in precipitation SO_4^{2-} from May 1979 to December 1987 consistent with SO_2 emission trends in upwind regions, and Clair et al. (1992) have shown an upward trend in ANC and pH for Nova Scotia and Newfoundland lakes over the period 1983–1989.

Because the networks used to monitor changes in surface water chemistry were established by different organizations with different priorities, no attempt has been made to conduct a unified evaluation of trends across southeastern Canada. Moreover, the regional studies that do exist typically used different statistical methodologies applied over differing time periods which complicates interregional comparisons. Sirois (1993) did use a single methodology to analyze trends in precipitation chemistry over eastern North America, but his work did not include a comparative analysis of trends in surface water quality. Hence the question remains as to whether these regional trends in precipitation chemistry are reflected in the chemistry of aquatic ecosystems.

Our work remedies these shortcomings by using a consistent statistical methodology to characterize trends in both precipitation and surface water chemistry over the period 1983–1991 at selected sites in Ontario, Québec, Nova Scotia, and Newfoundland. This facilitates intersite comparisons and evaluation of relationships that may exist between changes in atmospheric deposition and changes in surface water chemistry. We intend to produce a "snapshot" of how regional water chemistry has varied during a recent 9-year period over the large area of southeastern Canada that receives elevated levels of acidic deposition.

Study area

Water chemistry data were obtained for 111 sites: 11 stations in Ontario, 29 in Québec, 38 in Nova Scotia, and 33 in Newfoundland (Fig. 1). Precipitation chemistry data were taken from six CAPMoN (Canadian Air and Precipitation Monitoring Network) stations that spanned southeastern

Canada. They were located near water monitoring sites: Algoma (located at Turkey Lakes) and Chalk River in Ontario, Montmorency (located at Lac Laflamme) and Sutton in Québec, Kejimkujik in Nova Scotia, and Bay d'Espoir in Newfoundland (Vet et al. 1989).

The 11 Ontario water sites included two stream stations from the Turkey Lakes Watershed (TLW; Turkey Lake outflow (TO) and Batchawana Lake outflow (BO)) near Sault Ste Marie, seven streams from the Harp Lake catchment (HP; six inflows and one outflow), and two streams from the Plastic Lake catchment (PC; one inflow and one outflow) near Dorset. The TLW is situated on silicate bedrock (greenstones and granites) overlain by glacial tills that are generally <1–10 m thick. The soils are mostly ferrohumic and humoferric podzols that have a small carbonate content (0–2%), particularly at low elevations (Jeffries et al. 1988). Bedrock in the HP catchment is granitized biotite and hornblende gneiss, about 50% of which is covered by glacially deposited sandy and sandy loam tills varying from >1 to 10 m in thickness (Dillon et al. 1987; Scholer et al. 1990). The PC catchment has orthogneiss bedrock overlain by shallow (<1 m) sandy basal tills. The soils are mostly podzols or gleysols. Inflow streams drain wetlands that are either sphagnum–conifer swamps or shallow beaver ponds (Dillon et al. 1987, 1991; Dillon and LaZerte 1992). All Ontario sites are within the Great Lakes – St. Lawrence forest region.

The 29 Québec lakes chosen for this study included the Lac Laflamme calibrated watershed (LLW, "Montmorency" on Fig. 1) and 28 headwater lakes belonging to Environment Canada's Québec temporal monitoring network. The lakes are all on the Canadian Shield and situated in subregions 1–5 of the eight subregions defined by Bouchard (1992, 1993) for southern Québec. Bedrock geology is generally characterized by slowly weathering igneous rock (anorthosite, gabbro, granite, and syenite) and metamorphic rock (gneiss, paragneiss, amphibolite, and quartzite) except in the southwestern part of Québec, where the irregular occurrence of limestone, marble, and anorthosite leads to higher median ANC and Ca^{2+} and Mg^{2+} in the lakes (Dupont 1992). Soils are largely humoferric podzols. The lakes are situated in boreal forest (Bouchard's subregions 1–3) or mixed forest (subregions 4 and 5).

Seventy-one lakes from Environment Canada's Atlantic region monitoring network were selected for this study. Most of them are headwater lakes. Of the 38 Nova Scotia sites, 27 are located in Kejimkujik National Park (KEJ) and 11 are in the southwestern portion of the province near Yarmouth. The 33 Newfoundland lakes are more evenly distributed throughout the island (Fig. 1). For a more complete description of the Atlantic region network, see Clair and Esterby (1991) and Ehrman et al. (1993).

Geology of the Atlantic region is heterogeneous. Surficial geology in KEJ includes the slate-derived, well-drained sandy loams found in the Kejimkujik drumlins (Simmons et al. 1984), as well as granitic till and peaty organic deposits (Kerekes and Freedman 1989). Soils in the Yarmouth area are mostly shallow, stony, and sandy loams derived from quartzite and schist. A mixed forest type generally predominates throughout this part of Nova Scotia. Newfoundland geology includes greywacke, quartzite, gneiss, slate, schist, and granite (Howell 1986) overlain by coarse to medium textured glacial tills and humoferric podzols (Roberts 1983). Typical boreal forest occurs throughout the northern half of Newfoundland, while the south-central and southeastern part of the island is largely barren and dominated by dwarf shrub heaths, bogs, and fens. Many lakes in both Nova Scotia and Newfoundland are highly coloured due to drainage from organic deposits located in low relief, sphagnum-dominated basins.

Methods

Monthly data covering the period 1983–1991 were obtained for precipitation depth, average pH, and volume-weighted mean concentration and deposition of H^+, seasalt-corrected SO_4^{2-}, NO_3^-, and $Ca^{2+} + Mg^{2+}$ (Ca+Mg) from the Atmospheric Environment Service (AES) for the six CAPMoN stations (wet deposition only). A full description of the CAPMoN sampling protocol, analytical methods, etc., can be found in the annual summary reports (e.g., Vet et al. 1989).

Surface water samples were collected concurrently with a frequency of at least once per week (TLW, HP, PC, and LLW), six times per year (nine Québec sites), or twice a year during spring and fall overturn (remaining 20 Québec and all 71 Atlantic sites). Sampling protocols for Ontario sites, Québec network lakes, and Atlantic network lakes are documented in Locke and Scott (1985), Papineau (1987), Dubois et al. (1992), and Clair et al. (1992), respectively.

Samples collected at the TLW, Québec, and Atlantic sites were analyzed in Environment Canada laboratories for the full spectrum of major ions using standard procedures. Here, we will report results for pH (measured potentiometrically in the laboratory), ANC (Gran titration), SO_4^{2-} (ion chromatography or colorimetry if the sample colour was negligible), NO_3^- ($NO_3^- + NO_2^-$, also by ion chromatography or colorimetry), Ca+Mg (atomic absorption), and colour (Hazen procedure). HP and PC samples were analyzed at the Dorset Research Centre using similar methods (OME 1983). Applicable variables from Atlantic region lakes were corrected for the seasalt contribution (Clair et al. 1992).

Because coloured waters are common in Atlantic Canada (Ehrman et al. 1993), analyses of water in the Atlantic monitoring program routinely include dissolved organic carbon (DOC). DOC contains carboxylic functional groups which usually contribute to acidity so that at higher concentrations, the more naturally acidic the water. While DOC permits a more precise quantification of organic acidity than colour, a methodological change for DOC in the middle of the sampling period caused a shift in values which confused the statistical analysis. As the colour method remained the same throughout, we used this variable as a surrogate for DOC in the trend analysis. Should there be significant changes in colour, it may signify that changes in pH are due to natural rather than anthropogenic causes.

Prior to the statistical analysis, all data were validated by ion balancing, comparison of measured versus calculated conductivities, and outlier analysis. Both the federal and OME laboratories participated in Environment Canada's Long Range Transport of Air Pollutants interlaboratory quality assurance program.

Table 1. Decision tree used by DETECT to select which nonparametric test was used for trend analysis.

Persistence	Seasonality	Selected test	Reference
No	No	Kendall	Hirsch et al. 1982
No	Yes	Kendall seasonal	Hirsch et al. 1982
Yes	No	Spearman/Lettenmaier	Lettenmaier 1976
Yes	Yes	Hirsch and Slack	Hirsch and Slack 1984

Table 2. Summary of monotonic trends identified for precipitation depth, wet-only concentration, and deposition from six CAPMoN monitoring stations from eastern Canada for the period 1983–1991. The data were combined into 2-month intervals prior to statistical analysis. "EV" indicates a trend influenced by an extreme value.

Station	Precipitation depth	pH	H^+ concn.	H^+ dep.	SO_4^{2-} concn.	SO_4^{2-} dep.	NO_3^- concn.	NO_3^- dep.	Ca^{2+} concn.	Ca^{2+} dep.	Mg^{2+} concn.	Mg^{2+} dep.
Algoma	↔	↑	↓	↓	↓	↓	↔	↔	↔	↔	↔	↑
Chalk River	↔	↑	↓	↓	↓	↓	↔	↔	↓	↓	↔	↓
Montmorency	↔	↔	↔	↓	↔	↓	↔	↔	↔	↔	↔	↔
Sutton	↔	↔	↔	↔	↔	↔	↔	↔	↓	↔	↓	↔
Kejimkujik	↔	↔	↔	↔	↔	↔	↔	↑	↔	↔	↔	↔
Bay d'Espoir	↔	↔	↔	↔	↔	↔	↑	↑	↓ (EV)	↓ (EV)	↔	↔

Monotonic trend analyses were carried out on all precipitation and surface water variables using the DETECT software package (Cluis 1988; Cluis et al. 1989) and a null hypothesis of no change. DETECT chooses one method from a suite of nonparametric procedures depending on whether or not there is seasonality and (or) persistence in the data (Berryman et al. 1988). The methods and their selection criteria are summarized in Table 1. Seasonality is present when the means of predefined seasons (see below) are found to be different using an analysis of variance. Persistence, or serial correlation, represents a carryover of information from one observation to the next. It is unlikely to occur for water samples collected more than 1 week apart because of the highly dynamic nature of aquatic systems. Nevertheless, if DETECT identified statistical persistence, it was considered when choosing the method. A trend was significant when its probability of chance occurrence was below 5% ($\alpha = 0.05$). Trend magnitude was estimated using linear regression.

Early on, it was recognized that monotonic trend analysis of the Ontario SO_4^{2-} data might be problematic due to the effect of drought in several of the records (Dillon and LaZerte 1992) (e.g., see the record for Harp Lake inflow No. 4 (HP4) in Fig. 7, later in this paper). Therefore, a Mann–Whitney step trend procedure (Berryman 1984) that is also available as part of the DETECT package was applied to the 11 surface water records from Ontario.

Prior to running the analysis, DETECT allows the user to define seasonal intervals (e.g., monthly, bimonthly, quarterly, etc.). Monthly precipitation data and the data for Ontario and temporal Québec sites were grouped into bimonthly intervals. Data from spatial Québec and Atlantic sites were analyzed with a 6-month interval. If insufficient data were available to form a specified interval (e.g., due to missing data or removal of outliers), the missing interval was replaced with the mean seasonal value calculated for the whole time period. NO_3^- and colour values below the limit of detection (LOD) were replaced by a value equalling two thirds of the LOD.

Results

Precipitation quantity and chemistry in southeastern Canada

Among the six precipitation sites studied, Chalk River received the lowest precipitation amounts (≈80 cm/year), followed by Sutton, Algoma (≈130 cm), Montmorency, Kejimkujik (≈140 cm), and Baie d'Espoir (≈150 cm) (Fig. 2). With the exception of Sutton, 1987 was the driest year for every station. Most of the stations exhibited cyclical dry/wet year patterns. The exception was Chalk River where annual precipitation remained relatively constant throughout the study period. There were no significant trends in precipitation quantity at any site (Table 2).

SO_4^{2-} and H^+ deposition were greatest at the Ontario and Québec sites; the two Atlantic sites receive approximately half of that measured at Algoma and Sutton (Figs. 2 and 3). Significant increases in pH and decreases in H^+ deposition and concentration were observed for Algoma and Chalk River, while at Montmorency in Québec, only a decreasing H^+ deposition trend was found. Decreasing H^+ deposition was accompanied by decreased SO_4^{2-} deposition and (or) concentration at all three of these stations. No trends in precipitation H^+ or SO_4^{2-} concentration or deposition were observed at Kejimkujik or Baie d'Espoir.

The most common pattern in yearly SO_4^{2-} deposition exhibited by Algoma, Montmorency, and Bay d'Espoir was a reduction in deposition between 1983 and 1987 followed by a lesser, irregular increase. Chalk River showed a more steady decrease in SO_4^{2-} deposition over the period

Fig. 2. Annual precipitation (lines) and SO_4^{2-} deposition (bars) for the six CAPMoN stations. Note the differing vertical scales for deposition. SO_4^{2-} deposition in Atlantic Canada is approximately half that in Ontario and Québec.

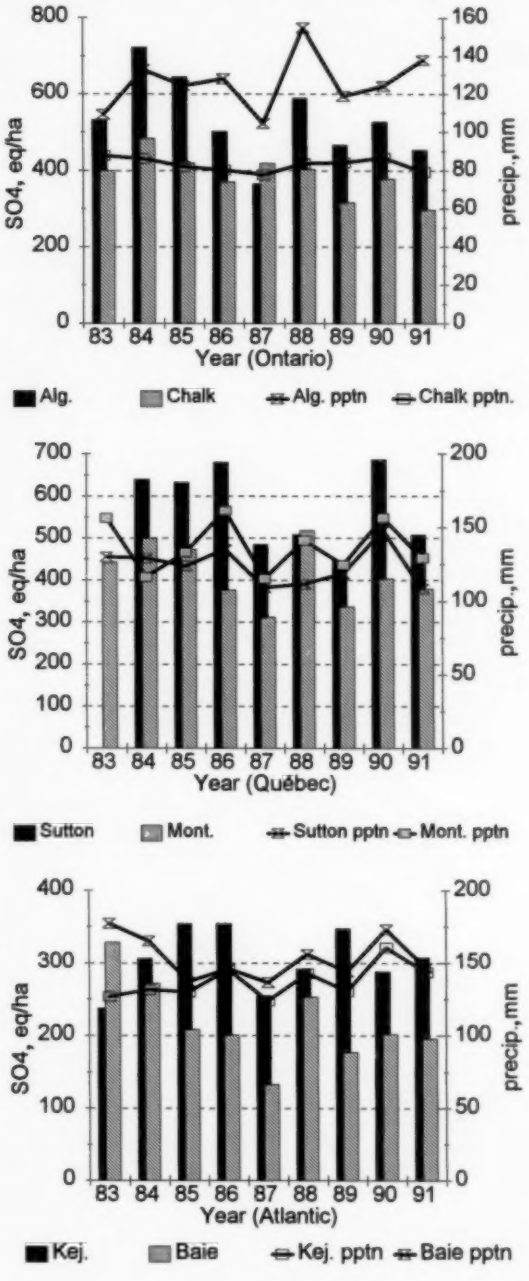
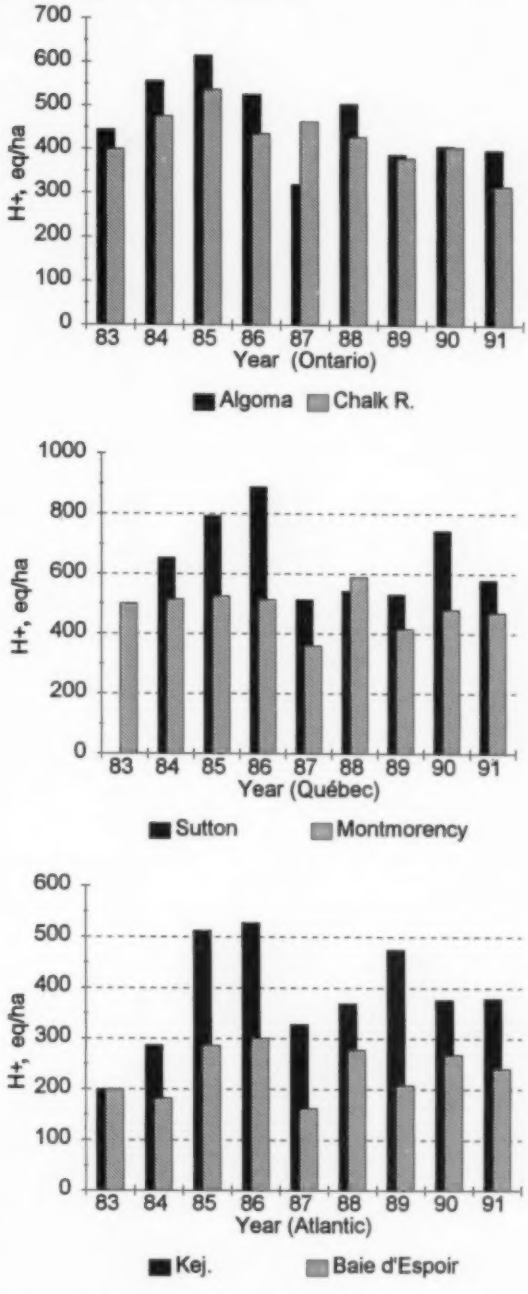
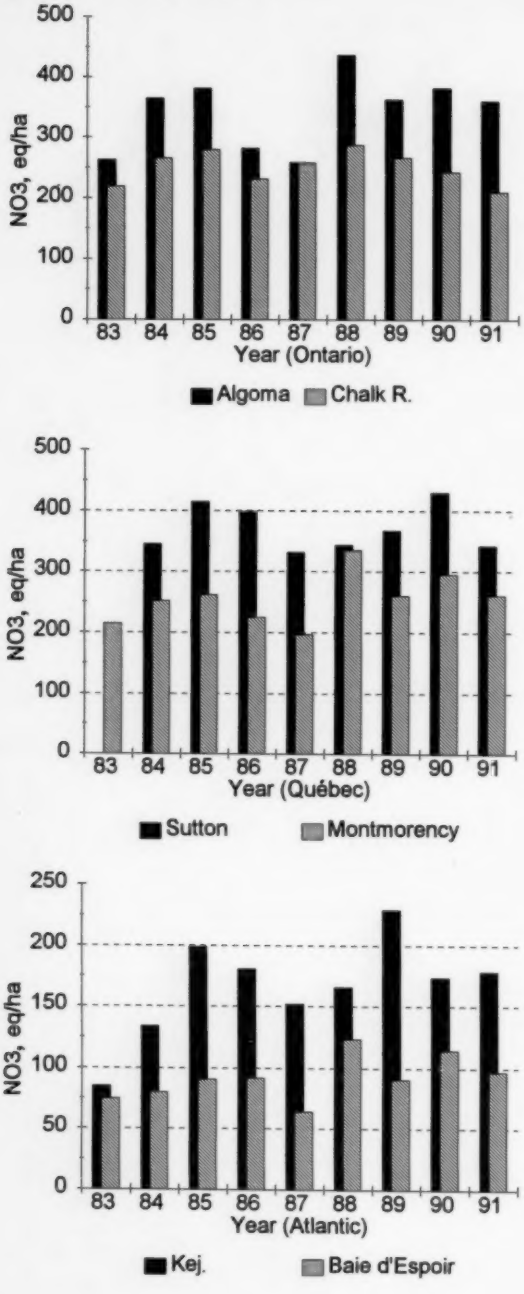


Fig. 3. Annual H^+ deposition for the six CAPMoN stations. Note the differing vertical scales.



1984–1991. Significantly decreasing SO_4^{2-} trends at Chalk River, Algoma, and Montmorency (Table 2) gave 1991 SO_4^{2-} deposition levels that were 26, 15, and 14% lower than those in 1983, respectively. SO_4^{2-} deposition at Sutton station exhibited a step change between the periods 1984–1986 and 1987–1991. At Kejimkujik, increases in SO_4^{2-} deposition between 1987 and 1989 gave levels at the end of the record that were almost the same as those in 1984; hence, the lack of detected trends for the entire period (Table 2).

Fig. 4. Annual NO_3^- deposition for the six CAPMoN sites. Note the differing vertical scales.



The temporal pattern of H^+ deposition followed that for SO_4^{2-} in Ontario and Nova Scotia (Fig. 3). The significantly negative trends in H^+ deposition found at Chalk River, Algoma, and Montmorency (Table 2) resulted in 1991 deposition levels that were 21, 10, and 6% lower than those in 1984, respectively. Significant increases in precipitation NO_3^- concentration were found at both Atlantic stations but not in Ontario or Québec (Fig. 4, Table 2).

With only one exception, precipitation base cation (Ca^{2+} or Mg^{2+}) concentration and deposition showed either no trend or a downward trend (Table 2). The decreasing trend in Ca^{2+} identified for Baie d'Espoir was influenced by one extremely high value in 1984 that may have been due to contamination. When this value was removed, no trend was detected.

Regional lake chemistry

The water quality of lakes sampled for this present study was compared with that reported by Jeffries (1991) for a much larger, regional subset of eastern Canadian lakes (Table 3). Our Nova Scotia and Ontario subsets were slightly more acidic (lower pH and ANC), while the Québec subset was less acidic than the larger set of lakes. As expected, lake SO_4^{2-} levels were highest in Ontario and lowest in Newfoundland, a pattern consistent with regional differences in deposition. SO_4^{2-} deposition is generally highest in southern Ontario, southern Québec, and southern New Brunswick, with lesser deposition occurring in regions west, north, and east of these areas (RMCC 1990). Highest Ca+Mg concentrations were found in Ontario.

In our data set, median NO_3^- was 11 μeq/L (range of station medians = 0.7–28) for Ontario and 1.4 μeq/L (range of station medians = 0.5–3.2) for Québec. Higher NO_3^- levels in Ontario compared with Quebec probably reflect relatively lower N retention in the TLW (Jeffries et al. 1986) and some HP watersheds (Dillon et al. 1991). Most NO_3^- concentrations in Atlantic Canada lakes were below the LOD.

Median colour in Nova Scotia and Newfoundland was 58 (sample range = 5–420) and 30 (range = 5–220) Hazen units, respectively. No colour data from Ontario and Québec were included in this study.

Lake chemistry trends

Analyses of data series using a 2-month sampling interval (i.e., all 11 Ontario sites and 9 of 29 Québec sites) found many cases of seasonality and persistence. In Ontario, all sites except HP6A exhibited seasonality for every variable, while persistence was found at nine sites for pH, SO_4^{2-}, and Ca+Mg and at approximately 50% of the sites for ANC and NO_3^-. Seasonality was common only for Ca+Mg and NO_3^- in the nine Québec lakes. Similarly, persistence was less prevalent in Québec than in Ontario, being found for eight of the nine lakes for pH and five of nine lakes for SO_4^{2-}.

All the remaining data series from Québec (i.e., for 20 lakes) plus those from Nova Scotia and Newfoundland used a 6-month sampling interval. Approximately half of the lakes in Québec and Newfoundland exhibited seasonality for ANC, with a higher proportion for Ca+Mg and much lesser proportions for the remaining variables. In Nova Scotia, approximately one third of the data series had seasonality for pH, ANC, Ca+Mg, and colour. The number of cases of persistence found in the data was surprisingly high given the twice-yearly sampling frequency. Occurrence of persistence was greatest (i.e., one third to one half of the lakes) for Ca+Mg in Québec and Newfoundland,

Table 3. Comparison of regional median pH, ANC, SO_4^{2-}, and Ca+Mg from this study and the larger regional database reported by Jeffries (1991). Regional abbreviations: ONT = Ontario, QUE = Québec, NS = Nova Scotia, NF = Newfoundland, SC ONT = south-central Ontario, NE ONT = northeastern Ontario. SO_4^{2-} and Ca+Mg are seasalt-corrected for NS and NF.

	This study				Jeffries 1991				
Parameter	ONT	QUE	NS	NF	SC ONT	NE ONT	QUE	NS	NF
pH	5.8	6.5	5.2	6.1	6.3	6.9	6.2	5.4	6.2
ANC, μeq/L	42	98	2	34	61	166	52	14	28
SO_4^{2-}, μeq/L	150	110	39	20	156	142	94	50	31
Ca+Mg, μeq/L	217	173	38	66	194	383	163	53	79

while a somewhat lower proportion was found for pH, ANC, and Ca+Mg in Nova Scotia. The relatively high incidence of persistence in our data probably is not environmentally relevant; however, it is important to keep persistence in mind when evaluating the identified trends, since it requires the use of lower powered tests (Berryman et al. 1988) that may fail to identify trends discernible by other methods.

Preliminary examination of the trend magnitudes showed little consistency among lakes, even those lakes that were spatially proximate. Moreover, significant trends for variables that should complement each other (e.g., pH and ANC) were not detected at the same site in some cases (see below). Given these observations, we consider quantitative assessment of trend magnitudes to be of limited value at this time. Therefore, although we will note a few trend magnitudes to give an impression of what was observed, we will emphasize the results for trend direction in the discussion that follows.

Ontario lake chemistry trends

The complexity of the changes observed in pH is illustrated by the responses of streams at Harp Lake. Two sites (one inflow and the outflow) exhibited increasing pH trends, while three other lake inflows showed decreasing pH trends. For the 9-year period, the magnitude of the greatest pH decrease was from 6.0 to 5.7 units at Harp Inflow No. 3 (HP3, Fig. 5), while the pH increases were 0.3 unit at both Harp Outflow (HPO, Fig. 5) and HP5. No pH trends were detected at TLW.

Three increasing ANC trends were found in Ontario: TO (from 56 to 66 μeq/L), HPO (from 51 to 69 μeq/L), and HP5 (from 28 to 77 μeq/L, Fig. 6). The trends observed at the two Harp Lake sites were consistent with the increasing pH trends. Similarly, the significant ANC decrease at HP6 (from 76 to 48 μeq/L) was consistent with a significant pH decline. However, the ANC trend at TO was not matched by a significant trend in pH, and the pH declines at HP3 and HP6A were not accompanied by significant change in ANC.

As noted above, SO_4^{2-} data from the Ontario sites were analyzed using a step trend procedure and they showed either no trend or increasing trends. Increasing trends were detected at five locations: three Harp Lake inflows (including HP4, Fig. 7), Plastic Lake inflow (PC1), and BO in the TLW.

Base cations (i.e., Ca+Mg) in Ontario remained unchanged (seven sites) or significantly increased (TO, HP4, HP6, and HPO), with magnitudes of the increases ranging from 17 to 190 μeq/L over the 9-year period (Fig. 8). Two of the sites with increasing Ca+Mg had increasing ANC (TO and HPO), one had increasing SO_4^{2-} and NO_3^- (HP4), and one had decreasing ANC and pH but increasing SO_4^{2-} (HP6).

NO_3^- increased at one Ontario site (HP4, from 107 to 124 μeq/L) and decreased at another (HP3, from 11.7 to 5.9 μeq/L); the remainder showed no significant NO_3^- trend.

Québec lake chemistry trends

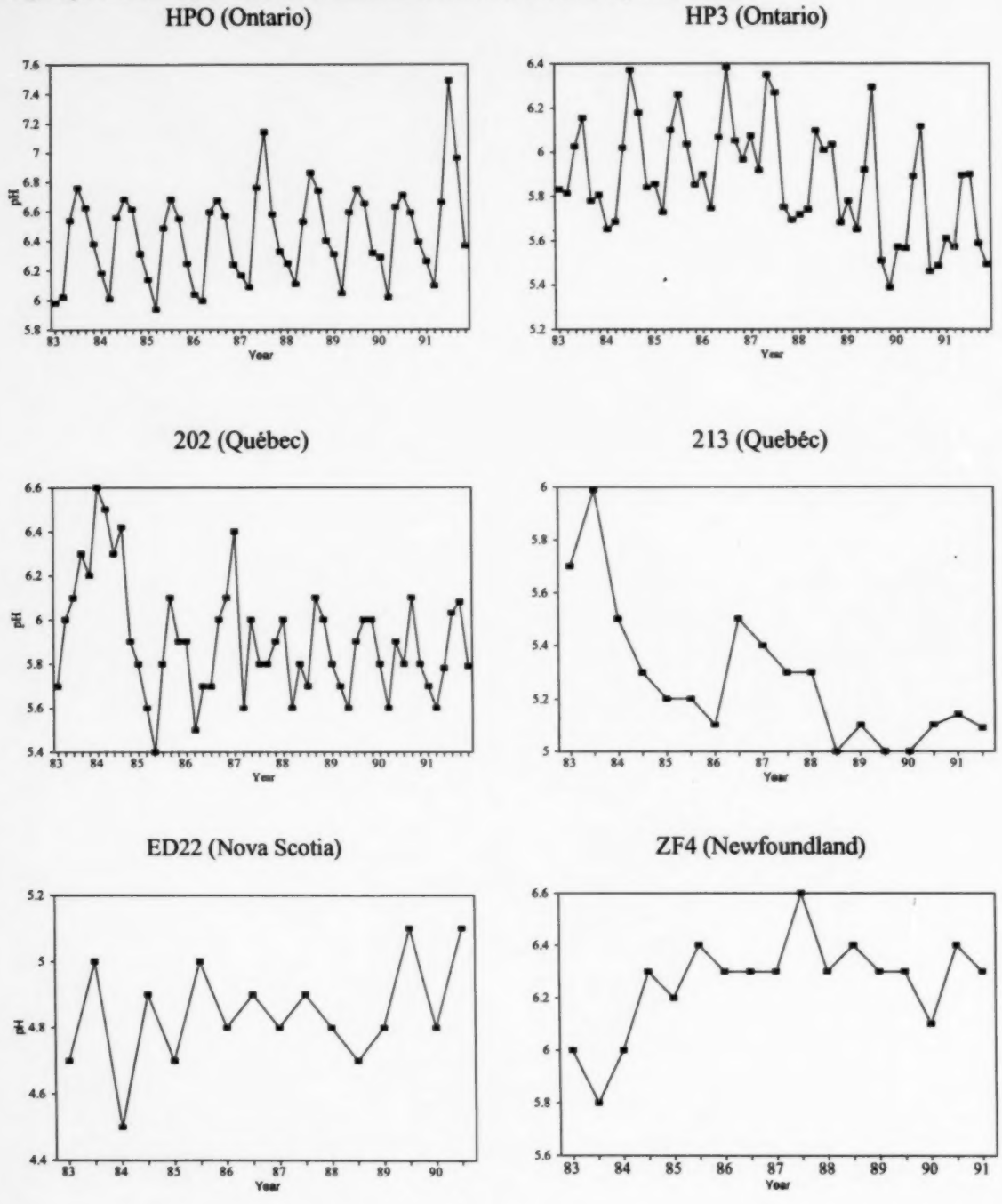
Five pH decreases were observed in the 29 Québec lakes, with no increases. The magnitude of the decreases ranged from 0.6 pH unit at Lake 213 (final pH = 5.0) to 0.3 unit at Lake 511 (final pH = 7.4). While there were no sites in Québec that had increasing ANC, compared with pH there was double the number of lakes (10) exhibiting significant ANC declines over the 9-year period (between 4.1 and 140 μeq/L).

Given the observed declines for pH and ANC, it was surprising that 14 lakes showed decreasing SO_4^{2-} trends, while only one had an increasing trend. The magnitude of the decrease was greatest for eight lakes in southwest Québec, ranging from 18 to 53 μeq/L. SO_4^{2-} decreases for the six lakes in other parts of the province ranged from 7.1 to 32 μeq/L. Several lakes also showed a reversal in the pattern of SO_4^{2-} concentrations, i.e., declining in the early 1980s and increasing in the late 1980s, thereby leading to no overall trend (e.g., Lake 101 in Fig. 7). Seven of the 14 Québec lakes showing SO_4^{2-} decreases also had significantly decreasing pH and (or) ANC. The remaining seven lakes had no associated pH or ANC trend.

Thirteen significant trends for Ca+Mg were detected in Québec, of which six were increases (from 4.7 to 27 μeq/L) and seven were decreases (from 7.5 to 161 μeq/L). Of the seven lakes with downward trending Ca+Mg, simultaneous trends for anions included: three lakes exhibiting ANC declines, two lakes in which both ANC and SO_4^{2-} decreased, and one with only SO_4^{2-} and another with only NO_3^- declining. Of the six lakes with upward trending Ca+Mg, three did not have accompanying trends for any other variables, two had both increasing SO_4^{2-} and NO_3^-, and one had only NO_3^- increasing.

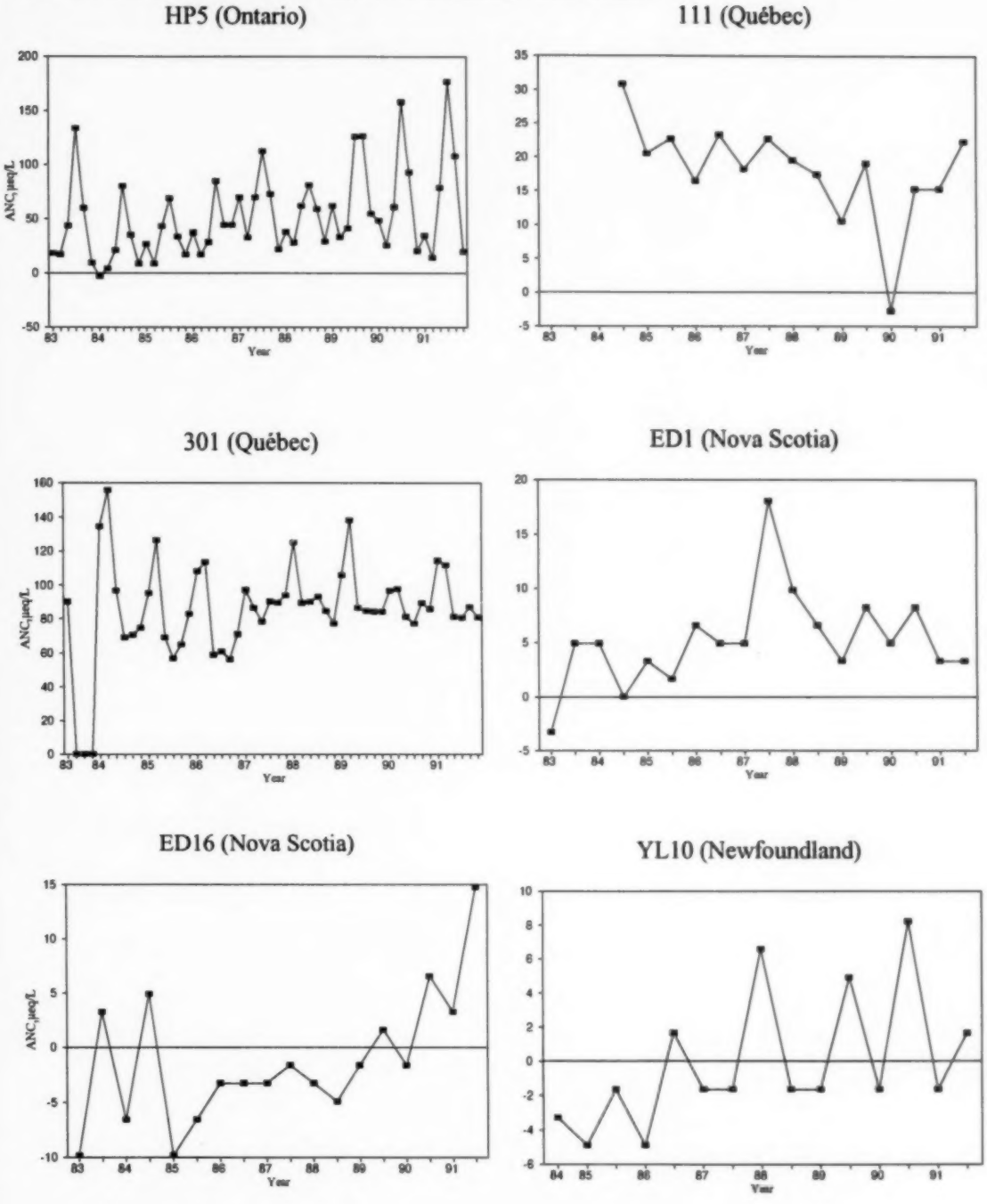
Québec was the only region where several significant trends in NO_3^- were detected. These included increases in

Fig. 5. pH time series plots for selected lakes and streams. Note the differing vertical scales.



11 lakes (ranging from 6 to 37 μeq/L over 9 years) and very small decreases in two lakes. These trends are consistent with the increasing concentration of NO_3^- in precipitation reported by Couture (1993) and Sirois (1993) at Montmorency for the period 1981–1989/90, although as reported above, our analysis of the complex pattern of NO_3^- in precipitation from 1983 to 1991 failed to detect a significant trend in Québec.

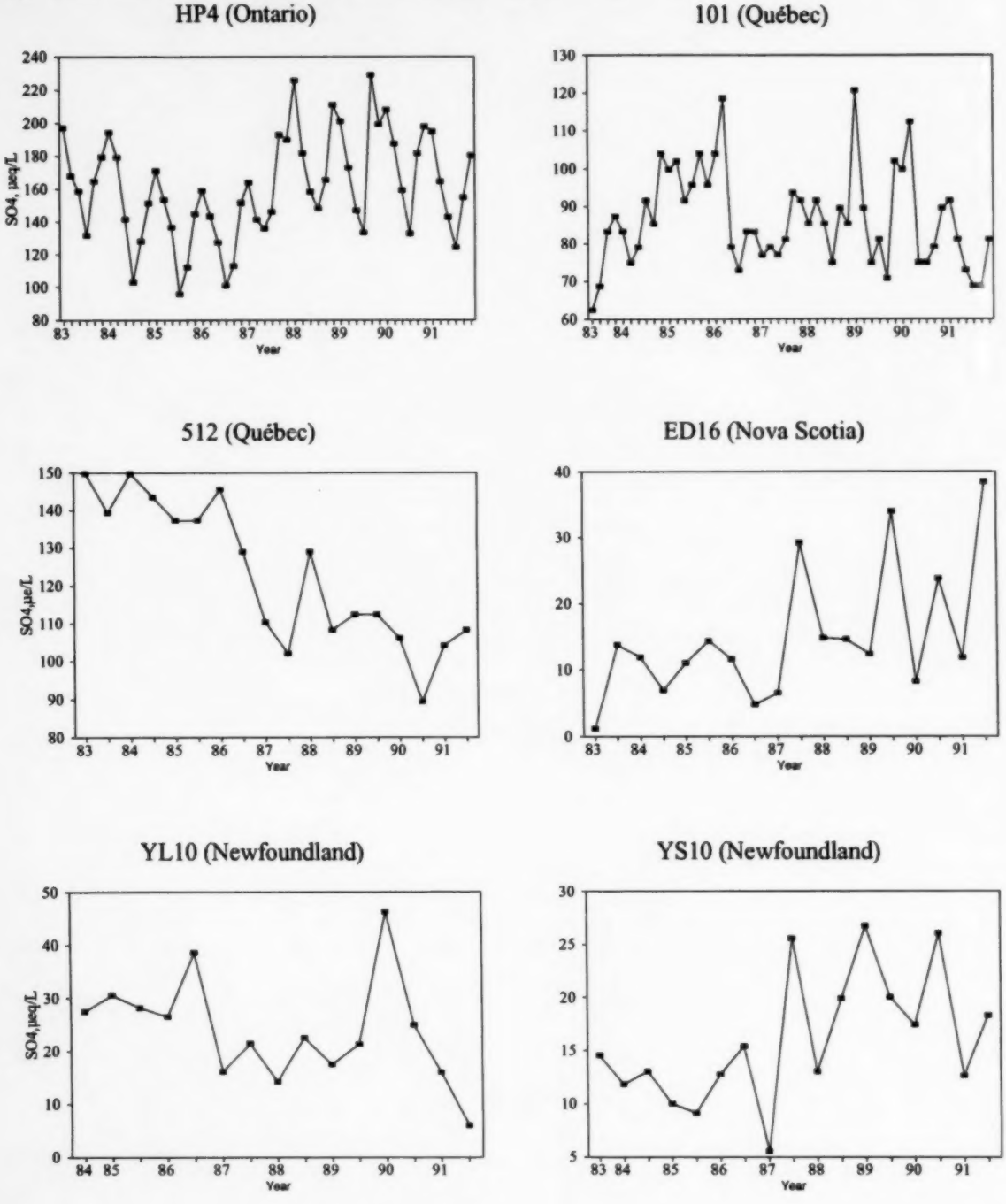
Fig. 6. ANC series plots for selected lakes. Note the differing vertical scales.



Atlantic region lake chemistry trends

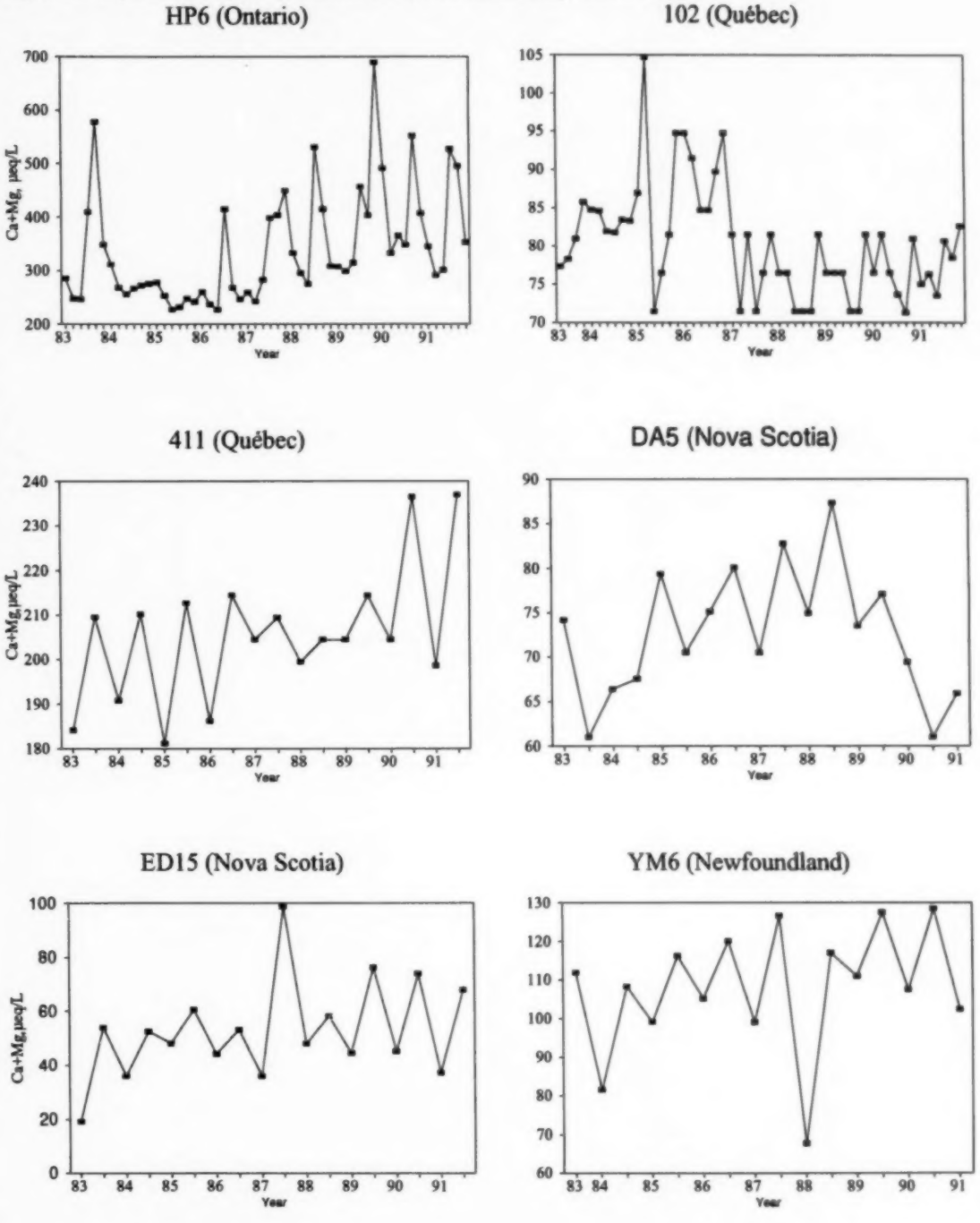
Over the 9-year period, there were three increasing pH trends (from 0.1 to 0.3 unit) and one small (<0.1 unit) decreasing trend in the 38 Nova Scotia lakes. They occurred in both the southwestern and south-central portions of the province. In Newfoundland, 4 of 33 lakes displayed small pH increases (all <0.1 unit), while one lake had a decreasing trend (0.2 unit with final pH = 5.5).

Fig. 7. SO_4^{2-} time series plots for selected sites. Note the differing vertical scales.



Lakes in the Atlantic region exhibited only positive or no trend for ANC. Fifteen of the 38 Nova Scotia lakes (located in both the Yarmouth and Kejimkujik areas) had upward trends, with the increases ranging from 4.3 to 18 µeq/L. The three lakes that exhibited increasing pH trends also had positive ANC trends, but so did the

Fig. 8. Ca+Mg time series plots for selected lakes. Note the differing vertical scales.



lake with decreasing pH. In Newfoundland, 16 of 33 sites showed positive ANC trends (increases from 3.8 to 22 µeq/L over the 9 years). Three of these lakes had corresponding pH increases.

There were 13 increasing SO_4^{2-} trends in Nova Scotia lakes, with magnitudes ranging from 5.8 to 18 µeq/L over the 9 years, and only one lake (EA2) showed a decreasing trend of 8.1 µeq/L. Of these 14 significant SO_4^{2-} trends,

seven also had increasing ANC, including Lake EA2. The predominantly positive direction of SO_4^{2-} trends from Nova Scotia therefore resembled that from Ontario.

There were six increasing and five decreasing SO_4^{2-} trends in Newfoundland. The magnitude of the increases varied from 8.1 to 14 μeq/L for the study period, while the decreases occupied a narrow range from 9.2 to 12 μeq/L. Five of six Newfoundland lakes with increasing SO_4^{2-} showed no change in pH and ANC, and the remaining lake had increasing ANC. In fact, most of the ANC increases (13 of 16) were not associated with any significant trend in SO_4^{2-}.

With Ca+Mg decreasing at two lakes and increasing at three, there was no general Ca+Mg trend in Nova Scotia lakes. Base cations increased in three Kejimkujik lakes (increases ranging from 5.4 to 22 μeq/L for the study period) and decreased in two others (one each from the Kejimkujik and Yarmouth areas). Similarly, there were only five significant Ca+Mg trends in Newfoundland although all were increasing (increases ranging from 8.6 to 21.8 μeq/L over 9 years).

Since most NO_3^- levels were below the LOD in Atlantic region samples, only three records (one in Nova Scotia and two in Newfoundland) were tested for trends. One of the Newfoundland sites was significantly increasing.

Increasing colour trends were detected in five Nova Scotia lakes although the results should be interpreted cautiously, since one trend was influenced by extreme values and another had a large number of values below the LOD. Six Newfoundland sites showed significant colour increases. These results suggest that natural acidity increased at these sites during the study period; however, a corresponding significant pH decrease was only recorded in one Newfoundland lake. There were five occasions where increasing colour overlapped with significantly decreasing SO_4^{2-}.

Discussion

Many statistically significant trends in the six lakes chemistry variables were identified across the four regions of southeastern Canada (Table 4). Some of the results may seem contradictory at first glance, e.g., 15 of 38 Nova Scotia lakes had increasing ANC but only 3 of 38 had increasing pH, while 13 had increasing SO_4^{2-}. However, it must be remembered that each of the trends was identified independently (e.g., only 6 of 38 Nova Scotia lakes had simultaneously increasing ANC and SO_4^{2-}) and that water chemistry is influenced by many factors that may affect trend detection, e.g., spatial and temporal inconsistency in deposition, interannual climatic variability, within-basin interactions such as wetland storage of S, or soil processes that transform N species, etc.

Our trend results for SO_4^{2-} in precipitation differed somewhat from those of Sirois (1993) although the overall interpretation is consistent. Sirois analyzed a slightly different time period (1979–1990) and used a more sophisticated statistical method (i.e., model fitting using least-squares regression). He identified decreasing trends in SO_4^{2-} concentration, not only at Algoma and Chalk River as we did, but also at Montmorency and Kejimkujik. Our more statistically conservative approach did not detect the decreasing SO_4^{2-} in precipitation that Sirois reported for the Atlantic region, although we did find significantly decreasing SO_4^{2-} deposition at Montmorency. Sirois did not analyze data from Sutton and Baie d'Espoir. Significant decreases in SO_4^{2-} deposition have also been reported for a number of sites in the central and eastern United States (and Sutton as well) for the period 1983–1988 (Newell 1993).

In the late 1970s to mid-1980s, declining SO_2 emissions over eastern North America were cited to explain decreasing SO_4^{2-} deposition at a number of CAPMoN stations (Dillon et al. 1988; RMCC 1990). Our observation that only half of the precipitation stations exhibited a significant downward trend in SO_4^{2-} deposition may reflect the slower rate of SO_2 emission reduction that occurred during the mid-1980s and early 1990s. The 1970, 1980, and 1990 SO_2 emissions were 5.6, 3.8, and 2.7 million tonnes, respectively, in eastern Canada (3.0 in 1985) and 31.2, 25.7, and 23.3 million tonnes, respectively, in the United States (Canada–US 1992).

Sirois (1993) also evaluated trends in precipitation NO_3^- concentration. Similar to our results, he found no NO_3^- concentration trend for Algoma and Chalk River, but differed by finding a steady increase at Montmorency and an increasing–decreasing pattern at Kejimkujik that peaked in 1988. Our analysis found an increasing trend in NO_3^- concentration only at Baie d'Espoir, although an increasing deposition trend was detected at both this and the Kejimkujik station. We did not confirm Sirois' NO_3^- increase at Montmorency which may be due to differences in statistical methodology or the time period analyzed.

Only 19 of our 111 surface water sampling sites showed significant pH trends (nine increases and 10 decreases, Table 4), implying an overall stability in water pH across eastern Canada; however, some regional differences exist. Surface waters near Dorset in south-central Ontario clearly show strong seasonal patterns for pH (Fig. 5), with nearly equal numbers of increasing and decreasing trends. In contrast, all five Québec sites exhibiting a significant pH trend were decreasing, while increasing pH trends predominated (seven of nine) at Atlantic region sites.

Considering all regions together, there were 34 increasing ANC trends (almost all in Ontario, Nova Scotia, and Newfoundland) and 11 decreasing trends (almost all in Québec). In most cases, complimentary pH and ANC trends were not detected in the same waters. This result is most likely a statistical artifact caused by differing relative levels of variability in the pH and ANC data records. Data variability strongly influences the detection of monotonic trends as attempted here, and in general, only longer monitoring records will allow identification of trends in "noisy" data. A second possible but less likely explanation is that the chemical components comprising ANC have changed also, e.g., bicarbonate exchanged for aluminium or organic anions. This could allow ANC to change but not pH. We do not have aluminium data to evaluate this possibility, but our colour data for the Atlantic region are a gross indicator of the organic anion concentration. Of the 11 significant colour trends (all increases), only four had concomitant ANC increases.

The glaciated terrain of southeastern Canada exhibits little within-basin retention of SO_4^{2-} (Rochelle et al. 1987). Therefore, one might expect to be able to interpret the

data in terms of a simple acid–base titration, i.e., increases or decreases in deposition lead to parallel changes in runoff SO_4^{2-} with appropriate compensatory changes in other ions. However, where we found trends for lake SO_4^{2-} (Table 4), they showed strong regional differences that were sometimes inconsistent with the trends in precipitation SO_4^{2-} (Table 2). Such trend inconsistencies probably arise because drainage basins are likely to need at least a few years to establish a new quasi-equilibrium with a changed SO_4^{2-} input. Lower precipitation quantities may lead to higher runoff SO_4^{2-} due to a concentration effect (Newell 1993), but our analyses found no trend in precipitation depth.

The situation in Québec seems to be the most straightforward example of a simple sulphuric acid titration, since the decline in deposition observed at Montmorency is concomitant with the predominantly negative trends (14 of 15) observed for lake SO_4^{2-}. Note, however, that there was no trend for precipitation SO_4^{2-} at Sutton, and ANC and pH trends in Québec lakes were generally unchanging or decreasing. An expectation underlying implementation of SO_2 emission control is that the decrease in runoff SO_4^{2-} will be chemically compensated by a reduction in acidity, i.e., an increase in pH and (or) ANC. At this time, such an effect is not widely manifest in Québec. Similar decreases in lake SO_4^{2-} were reported for the 1980s in the northeastern United States that were consistent with decreasing SO_4^{2-} deposition (Driscoll et al. 1991; Kahl et al. 1991, 1993), but like our Québec data, Driscoll et al. (1991) also reported decreasing ANC levels. Increases in NO_3^- may account for at least some of the lack of improvement of ANC and pH observed in both Québec and northeastern United States waters. This situation is similar to that found in southern Norway where the regional acidity status has remained relatively constant despite reductions in SO_4^{2-} deposition and runoff (Henriksen et al. 1988).

Increasing SO_4^{2-} trends in Ontario surface waters were not consistent with the decreasing trend identified in deposition (Table 2). This may be related to climatic factors. The discontinuity in the SO_4^{2-} records for Ontario waters (e.g., HP4, Fig. 7) that lead to application of the step trend procedure was contemporaneous with the driest year of the 9-year period (i.e., 1987, see Fig. 2). There have been similar observations in other parts of Ontario. For example, Keller et al. (1992) reported that SO_4^{2-} increased in Sudbury region lakes between 1987 and 1989 despite relatively stable local SO_2 emissions over the latter half of the 1980s. They attributed this observation to the leaching of stored SO_4^{2-} from watersheds and wetlands and to mobilization of S from littoral sediments in one particularly wet year (1988) following two consecutive dry years. Trends for other chemical variables in Ontario were not systematically related to the SO_4^{2-} trends. It is likely that some basins respond to reduced SO_4^{2-} inputs by decreasing cation export rather than increasing ANC, a response noted in Algoma area lakes by Kelso and Jeffries (1988).

Like Ontario, trend interrelationships were very irregular in the Atlantic region. In Newfoundland, for example, only one of the six increasing lake SO_4^{2-} trends was associated with a significant trend in ANC (also increasing), while three of the five cases of decreasing SO_4^{2-} corresponded with two increasing ANC trends and one decreasing pH trend. In Nova Scotia, about one half of the lakes with SO_4^{2-} increases also showed ANC increases. Although the SO_4^{2-} records for Atlantic region lakes do not show the pronounced concentration discontinuity observed in Ontario around 1987, they do suggest that for at least some stations (e.g., two of three lakes in Fig. 7), increased between-sample variability in SO_4^{2-} occurred after this date. Despite the increasing NO_3^- deposition found for both Atlantic region precipitation stations, extreme nutrient impoverishment in many lakes means that this input NO_3^- is acting more as a fertilizer than an acidifier.

It is evident that the trend in acidification status of lakes in southeastern Canada cannot be evaluated using the straightforward analogy of an equilibrium acid–base titration. Comparing temporal changes in the surface water chemistry of many of our monitoring basins with changes in deposition shows that there is not a consistent equilibrium relationship between the two, and in fact, geochemical equilibrium seems to be rare under present conditions. This may be due to the rather large changes in SO_4^{2-} deposition

Table 4. Summary of variable trends by region. Due to many NO_3^- values below the LOD, trend analysis for this variable was only possible for one Nova Scotia and two Newfoundland lakes.

	↑	↓	↔
pH			
Ontario	2	3	6
Québec	0	5	24
Nova Scotia	3	1	34
Newfoundland	4	1	28
ANC			
Ontario	3	1	7
Québec	0	10	19
Nova Scotia	15	0	23
Newfoundland	16	0	17
Sulfate			
Ontario	5	0	6
Québec	1	14	14
Nova Scotia	13	1	24
Newfoundland	6	5	22
Ca+Mg			
Ontario	4	0	7
Québec	6	7	16
Nova Scotia	3	2	33
Newfoundland	5	0	28
Nitrate			
Ontario	1	1	9
Québec	11	2	16
Nova Scotia	0	0	1
Newfoundland	1	0	1
Colour			
Nova Scotia	5	0	33
Newfoundland	6	0	26

Table 5. Classification of surface water acidification status by region. The classification is based on pH and/or ANC trends (see text).

Class	Status	Ontario	Québec	Atlantic	Total
1	Stable	5	16	39	60
2	Acidifying	3	13	1	17
3	Improving	3	0	31	34
	Total	11	29	71	111

that have occurred over the study period (i.e., the interannual variations, multiyear patterns, or consistent trends; Fig. 2). Undoubtedly, wetlands are playing an important role in SO_4^{2-} retention-export dynamics (Dillon and LaZerte 1992), especially for central Nova Scotia and Newfoundland lakes.

Our results are influenced by the time "window" used in the analysis. For example, Clair et al. (1992) used the same statistical approach and Nova Scotia lakes and found mostly significant decreases in SO_4^{2-} for the period 1983–1989. With the addition of two more years of data, we have found mostly significant SO_4^{2-} increases. In Newfoundland, Clair et al. (1992) found that SO_4^{2-} trends were similar to those in this study but they reported more increasing pH trends (15 vs. 4) and fewer ANC trends (6 vs. 16) than reported here. Even our 9-year data set has a limited ability to identify "real" as opposed to "statistical" long-term trends. Continuation of the monitoring program is essential.

Based on the pH and ANC trends detected in lakes between 1983 and 1991, we divided our monitoring stations lakes into three classes: Class 1, where pH and ANC showed no trends, Class 2, where pH and/or ANC were decreasing, and Class 3, where pH and/or ANC were increasing (Table 5). Class 1 stations were considered to have a stable acidification status. Class 2 waters are acidifying, while Class 3 implies recovering or improving situations. Our classification scheme is based on generous criteria, since many combinations of trends in acidity indicators (pH and ANC) with other chemical trends may still fall within a single class.

Interpreting acidification status based solely on ANC trends must be done with caution. Bricker and Rice (1989) and Kilham (1982) showed that increases in acidic deposition in basins containing carbonate geology can lead to increases in surface water pH, ANC, and base cations (Ca+Mg) by promoting $CaCO_3$ weathering and generating alkalinity. Most basins in our monitoring network have highly resistant silicate bedrock and surficial materials that do not act in themselves as a source of ANC during weathering. In these basins, ANC and pH are therefore expected to rise during deacidification, while base cations show little change because a decrease in aluminium will be the primary cation response (Schnoor and Stumm 1986). The most likely exceptions occur in central Ontario (as noted above), where small amounts of $CaCO_3$ in the glacial tills can influence surface water chemistry (Jeffries 1991).

Keeping these reservations in mind, our very simple classification scheme categorized 5 of 11 Ontario sites as stable (BO, PC1, PCO, and 2 Harp Lake inflows), three as acidifying (all Harp Lake inflows), and three as recovering (TO, HPO, and HP5). For the period 1979–1985, Dillon et al. (1987) reported stable conditions in Harp Lake (i.e., stable pH and ANC) and acidification in Plastic Lake (i.e., decreasing pH, ANC, and base cation trends; stable SO_4^{2-}). Thus, in contrast with the earlier period, a number of Harp Lake sites changed between 1983 and 1991, while Plastic Lake sites now seem to be stabilizing.

The restricted geographical coverage provided by our Ontario sites prevents spatial generalization of the trend results. However, a number of recent studies help to remedy this shortcoming. Kelso et al. (1992) reported the recovery of lakes from the Algoma area between 1979 and 1988 (i.e., increasing pH and decreasing SO_4^{2-} and Ca^{2+}). Keller et al. (1992) also reported improving lake chemistry in the Sudbury area during the late 1970s to the mid-1980s followed by a subsequent levelling off or even increasing acidity at some sites in the late 1980s. The available data suggest that local deposition patterns and regional acidification histories must be considered when attempting to explain the mixed results from Ontario.

Despite the fact that decreasing trends in water SO_4^{2-} predominated in Québec, all 29 lakes fell into Class 1 or 2, 16 were stable, and 13 were acidifying. However, three lakes in the acidifying class had median pH > 7.0 and median ANC > 200 μeq/L and thus are not considered sensitive to acidification.

The Atlantic region results contrast with those in Québec, since increasing SO_4^{2-} trends predominates in the surface waters, yet there is a greater tendency towards higher pH or ANC. Of the 71 Atlantic region lakes, 38 were stable, 31 improving, and only one lake was classified as acidifying. One lake did not fit into our classification scheme because it had both increasing ANC and decreasing pH.

The results of our trend analyses for eastern Canada illustrates the variable influence exerted by geography, geology, and deposition on water chemistry. Although SO_4^{2-} deposition seems to be decreasing over much of eastern Canada, landscape type, the nature of soils, their parent material, vegetation, and also climate combine to complicate the ecological response.

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Use of line transect methods to estimate abundance of benthic stream fishes

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Abstract: Visual estimation of stream salmonid abundance using strip transect sighting models has become commonplace. Application of visual estimation to other stream fishes, particularly benthic forms, has been limited. Examination of the distribution of sighting distances for the Roanoke darter (*Percina roanoka*), Roanoke logperch (*P. rex*), and black jumprock (*Moxostoma cervinum*) indicates that strip transect sighting models that assume probability of sighting remaining constant out to the limits of observer visibility are not appropriate for these benthic species. Our datasets indicate that distance sampling models that assume decreased sighting probability with increasing distance of the target from the observer provide a reasonable alternative to strip transect sighting models. There was a strong positive correlation between abundance estimates calculated using two alternative distance sampling models, as well as between the distance sampling model estimates and an estimate of abundance obtained with a backpack electroshocker.

Résumé : L'estimation visuelle de l'abondance des salmonidés dans les cours d'eau à l'aide de modèles des observations sur des transects en bandes est devenue une pratique courante. L'application de l'estimation visuelle à d'autres poissons lotiques, particulièrement des formes benthiques, est toutefois limitée. L'examen de la distribution des distances d'observation des dards *Percina roanoka* et *P. rex* et du suceur *Moxostoma cervinum* indique que les modèles d'observation sur transects en bandes qui posent que la probabilité d'observation reste constante jusqu'aux limites de visibilité de l'observateur ne sont pas appropriés pour ces espèces benthiques. Nos séries de données indiquent que les modèles d'échantillonnage à distance qui posent une réduction de la probabilité d'observation avec l'accroissement de la distance entre la cible et l'observateur constituent une alternative raisonnable aux modèles d'observation sur transects en bandes. Il y avait une forte corrélation positive entre les estimations de l'abondance calculées à l'aide de deux modèles différents d'échantillonnage à distance, ainsi qu'entre les estimations des modèles d'échantillonnage à distance et une estimation de l'abondance obtenue à l'aide d'un appareil portatif de pêche électrique.
[Traduit par la Rédaction]

Introduction

Underwater observation is well established in the Pacific Northwest as a method of estimating distribution and abundance of salmonids (Northcote and Wilkie 1963; Schill and Griffith 1984; Slaney and Martin 1987). It has also been used successfully in surveys of abundance of lacustrine (Keast and Harker 1977; Turner and Mackay 1985) and marine reef species (Sale and Douglas 1981; Thresher and Gunn 1986) and has proven to be efficient, reliable, precise, and accurate. Where comparisons with traditional methods (e.g., depletion estimation, mark–recapture, use of toxicants) have been carried out, underwater observation often performs as well or better than the traditional techniques, with a signifi/ ant reduction in effort.

Use of underwater observation for surveys of benthic species has been limited, and information on the precision and reliability of estimates is lacking. The fact that underwater

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[1] The Unit is jointly sponsered by the U.S. Fish and Wildlife Service, the Virginia Department of Game and Inland Fisheries, the Virginia Polytechnic Institute and State University, and the Wildlife Management Institute.

observation is not a common technique for assessing benthic species abundance may be due to assumptions associated with its more usual application to large, highly visible species in waters of good clarity. Most of these studies assume that all fish in a strip of set width are visible, and therefore, a total count is possible for that strip. Density is estimated by dividing the number of fish counted by the area sampled (Keast and Harker 1977; Schill and Griffith 1984; Turner and MacKay 1985), analogous to the strip transects used in wildlife studies (Seber 1982). In some instances, visual counts have been adjusted using correction factors based on estimates obtained with alternative estimation techniques (i.e., three-pass depletion estimates) under similar conditions. In contrast with many of the highly visible water column species typically assessed with direct observation, benthic species are often small, cryptically colored forms living in complex habitats where it may not be possible to designate a strip where all targets are seen with certainty.

An alternative to strip transect estimation is to use a group of models based on distance sampling (Eberhardt 1968, 1970; Burnham et al. 1980; Buckland et al. 1993), specifically those based on line transects. Although used in wildlife studies for over 40 years, and more recently in the study of marine mammals, these models have only sporadically been applied to studies of fish populations (Thresher and Gunn 1986; Bergstedt and Anderson 1990). Rather than assuming that the probability of seeing a target is 1.0 out to the limits of observer visibility, line transect models assume that only those targets near the observer are seen with certainty. Then, as the distance between the target and the observer increases, the probability of seeing the target decreases. The central problem with these models is estimation of a function that adequately characterizes sighting probability as distance from the observer increases.

Our objectives in this study are to (1) determine whether line transect or strip transect models provide an appropriate conceptual approach for the estimation of abundance of benthic stream fishes, (2) compare estimates of abundance calculated from two different line transect sampling models, and (3) compare the estimates obtained from the line transect models with those obtained using two traditional methods, electrofishing and strip transects.

Methods

Study area and study species

The study was conducted on the Roanoke River in Roanoke County and the South Fork Roanoke River in Roanoke and Montgomery counties, Virginia. Five sites were sampled on the Roanoke River during August, September, and October 1991 and nine sites were sampled on the South Fork Roanoke River during August and September 1992. As the three target species for this study were benthic riffle fishes, each of the sites included at least one full riffle-run sequence. Most of the sites also included the tail and head of the adjoining upstream and downstream pools. Study sites ranged in length from 130 to 168 m and in average width from 13.5 to 24.7 m.

The three species exhibit behavioral and morphological differences. The Roanoke darter (*Percina roanoka*) is small (40–70 mm TL), cryptically colored, relatively solitary, and tends to remain stationary when approached by a snorkeler. Movements are usually in short, rapid bursts. The Roanoke logperch (*P. rex*) is similar to the Roanoke darter, but is larger (60–140 mm TL) and less tolerant of snorkelers. The black jumprock (*Moxostoma cervinum*) is larger still (70–220 mm TL), uniformly colored, tends to move continuously, and occurs either singly or in groups of up to 40 individuals. Of the three study species, the black jumprock is the most difficult to approach without disturbance.

Underwater observation

Ensuring that observers' passage through the study area is along a well-defined line of travel and that measurements of distance from that line to the location of observed targets are both precise and accurate are key concerns in line transect studies. To meet these criteria, parallel lines oriented along the flow of the river were marked by yellow nylon twine on the day of sampling. Between two and five lines were located at each site depending on the width of the area to be sampled. Spacing between lines was based on water clarity and was a minimum of 1.5 times maximum visibility on the day of sampling. Maximum visibility was determined in one of the two ways. During 1991, a Secchi disk was suspended vertically in the water column in front of a snorkeler. The snorkeler moved away from the disk until it was no longer visible. The distance between the snorkeler and the disk was measured and served as the maximum visibility for that day. There was a strong correlation ($r^2 = 0.99$, $P < 0.001$) between maximum visibility and turbidity, measured in nephelometric turbidity units (NTU) by a Hach model 940 turbidimeter. During 1992, turbidity measurements served as a surrogate for maximum visibility.

Following placement of the transect lines, snorkelers entered the water downstream of the area to be sampled and one snorkeler moved slowly upstream along each of the lines, keeping the centerline of the body over the nylon twine. Each observer scanned the stream bottom directly in front and to both sides of the line of travel. When one of the three target species was sighted, a weighted marker was placed on the stream bottom at the location where the fish was first spotted. Markers were color-coded by species and numbered to allow identification of markers from different passes on the same day. Given that the spacing between lines resulted in some overlap between transects, double counting of fish was avoided by having adjacent observers remain abreast of each other. When a fish was observed in an area of potential overlap, communication between observers ensured that only one marker was placed for that fish. Depending on time, weather, and availability of personnel, one to eight complete passes (a single pass encompassing snorkeling each of the individual lines one time) were made through each site. On days when more than one pass was made through a site, a minimum of 1 h separated the completion of one pass and the initiation of the next. This "rest period" allowed fish to recover from disturbance caused by the preceding snorkeling pass. Upon completion of all snorkeling passes on a given day, the perpendicular distance of each marker from the transect line, species, transect number, and pass number were recorded.

Electrofishing

We also used a quadrat-based electrofishing technique with a Coffelt BP-6 AC backpack electrofishing unit and a seine 4.5 m long, 1.5 m deep, with mesh size of 0.6 cm, and a 1.5 × 1.5 × 1.5 m bag attached. The location of quadrats at each site was determined systematically. Quadrats were approximately rectangular with a width of 4 m and length of 10 m, giving a total area sampled in each quadrat at 40 m^2. Starting at the downstream end of each site, sampling proceeded upstream along a series of transects placed perpendicular to the streamflow at 15-m intervals. The first quadrat on each transect was placed at least 2 m from the adjacent bank. The adjoining edges of quadrats along the same transect were at least 2 m apart. The number of quadrats along a given transect was a function of stream width at that transect and the criteria previously mentioned. Upstream and downstream limits for quadrats on a given transect were marked by placing fluorescent tape on the stream bank at the appropriate point, while the center of a quadrat was identified by placing a weighted fluorescent marker on the stream bottom.

Following location of the quadrat, the seine was held downstream of the area to be sampled. Once the net was in place, the area immediately upstream of the net was thoroughly shocked. Following completion of the electrofishing pass, the net was lifted and all target species retained in the net were identified and counted. The area shocked was visually inspected to ensure that all stunned fish had been carried into the net. Fish remaining on the stream bottom within the quadrat were retrieved and included in the sample. In all cases, personnel avoided wading through quadrats prior to sampling. Spacing and location of quadrats and techniques used during setting and lifting of the bag seine were designed to minimize disturbance of areas to be shocked.

Density estimation

As stated earlier, the central problem in distance sampling is the specification of the form of the sighting function. Numerous models, varying in their underlying assumptions, have been developed to address this issue (reviewed in Buckland et al. 1993). At one extreme are models that make few assumptions about the shape of the sighting function. The distribution of sighting distances is used to fit the best function for the data. These models are statistically robust, but reliable estimates of the sighting function can only be obtained with fairly large sample sizes.

At the other extreme are conceptually simple models that depend on restrictive assumptions about the shape of the sighting function. Although such models require fewer observations, minor violation of the underlying assumptions may result in markedly biased estimates. Furthermore, these simpler models lack the strong statistical foundation of the data-intensive models, and estimation of their precision (i.e., variance) is problematic. The models used in this study represent the two extremes of the potential models.

The first model we used a line transect approach developed by Burnham et al. (1990) that uses the Fourier series (Crain et al. 1979) to estimate density. It makes few assumptions about the shape of the sighting function. The formula for density estimation is

$$[1] \quad D = n \cdot (f(0)/2L$$

where L = length of line sampled (metres), n = number of each target species detected, and $f(0)$ = function $f(x)$ evaluated at 0 distance from the transect line. The function $f(x)$ is the estimated probability density function for the target species evaluated at distance = x.

Robust estimation of $f(0)$ requires a minimum of 40 observations, and sample sizes greater than 80 are preferable. Few of the site/species combinations in this study yielded observation numbers of that magnitude, so it was necessary to pool observations across sites to obtain valid estimates of $f(0)$. Pooling of observations across sites is acceptable if it can be assumed that conditions influencing the shape of the sighting function are consistent across the pooled sites (Buckland et al. 1993). Experience during sampling indicated that both fish behavior and observer efficiency varied as a function of turbidity levels, so sites were divided into three groups based on turbidity levels on the day of sampling and an estimate of $f(0)$ developed for each species/turbidity level combination. Low-turbidity sites were those with NTU readings less than 3.5, moderate-turbidity sites were those with NTU readings from 3.5 to 5.0, and high-turbidity sites were those with NTU readings greater than 5.0. Pooling of sites in this manner yielded sample sizes for each of the turbidity level/species combinations greater than 40, with the exception of Roanoke logperch at high-turbidity sites where the sample size was only 29. The datasets were further modified prior to modelling of $f(0)$ by excluding the upper 5% of observations from each of the species/turbidity classes. These upper outliers have little if any effect on the final density estimate, and their exclusion leads to lower variance and better model fit for the function $f(0)$ (Burnham et al. 1980; Buckland et al. 1993). Estimates of the sampling variance of each of the site/species combinations were obtained using the equation

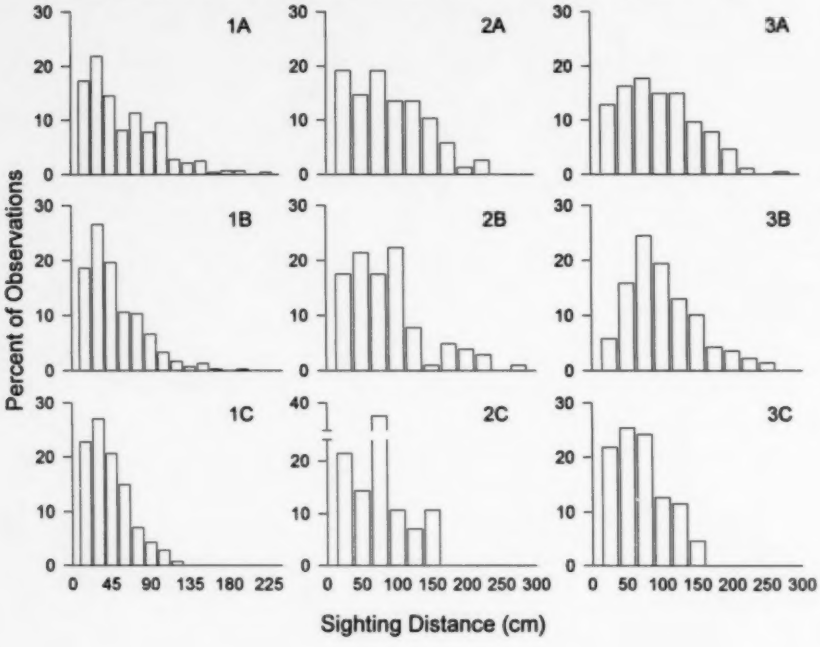
$$[2] \quad \text{var}(D) = D^2 \, [\text{CV}(n)^2 + \text{CV}(f(0))^2]$$

where CV = coefficient of variation (100·SE/mean).

For all sites, the number of replicate lines was less than or equal to five, yielding poor estimates of var(n), so it was assumed that var(n) = 2n (Burnham et al. 1980). Log-based 95% confidence intervals were calculated for each of the site/species density estimates. All calculations were carried out using the program DISTANCE (Laake et al. 1993).

The second density estimation model is based on an approach popularized by Emlen (1971) for surveys of songbirds. The Emlen model assumes that the probability of seeing a target is equal to 1.0 out to some minimum distance from the observer. Beyond this minimum distance, sighting probability declines out to some boundary width beyond which no targets are observed. Given this assumption, a smoothed frequency distribution of sighting distances should exhibit a flat shoulder proximal to the y-axis. The point at which the frequency distribution begins to dip defines the distance at which sighting probability begins to decline. The number of observations in the proximal strip where sighting probability is assumed to equal 1.0 is taken as an estimate of the true density of the target organism. A coefficient of detectability (CD) is calculated using the equation

Fig. 1. Frequency distributions of perpendicular sighting distances for (1A–1C) Roanoke darter (2A–2C), Roanoke logperch, and (3A–3C) black jumprock under (1A–3A) low-turbidity (NTU < 3.5), (1B–3B) moderate-turbidity (NTU ≥ 3.5 and NTU < 5), and (1C–3C) high- turbidity (NTU ≥ 5) conditions. Note the break in the *y*-axis for 2C.



[3] $CD = n_1 \cdot (W_2/(n_2 \cdot W_1))$

where W_1 = outer boundary width, W_2 = width of area in which sighting probability is assumed to equal 1.0, n_1 = total number of organisms sighted, and n_2 = number of sightings in W_2. The density of organisms in new areas is then

[4] $D = n/(2 \cdot L \cdot W_1 \cdot CD)$

where n = number of sightings in the area where density is to be estimated and L = length of line snorkeled.

For our calculations, a cumulative frequency plot (cfp) of cumulative number of detections versus strip width was constructed. W_2 and n_2 were obtained by determining the line through the origin that was tangent to the cfp and then setting W_2 and n_2 equal to the strip width and cumulative number of detections, respectively, at the point where that line was tangent to the cfp (equivalent to the distance where the probability of sighting no longer equals 1.0). The outer boundary width (W_1) was set equal to the width corresponding to the 95th percentile for the sighting distances. As with the Burnham estimates of $f(0)$, individual CD values were calculated for each turbidity level for each species.

Densities were also calculated using a strip transect model. For each site, strip width was set equal to twice the maximum visibility on the day of sampling, as determined by either Secchi disk extinction or turbidity level. The length of line snorkeled was multiplied by the strip width to estimate the area sampled. The number of sightings for each species was divided by the area sampled to obtain the density estimate for that species and site.

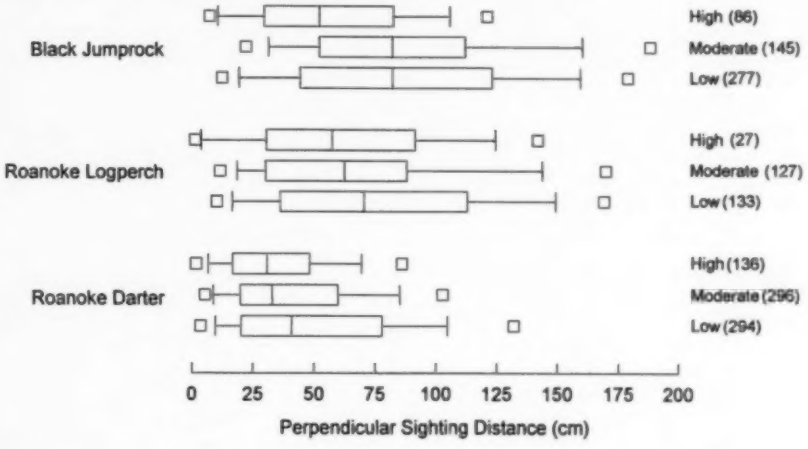
Densities for the electrofishing samples were calculated by multiplying the number of quadrats shocked at each site by 40 m^2, giving the total area sampled. The number of each species collected from all quadrats was divided by the total area sampled to obtain an estimate of the number of each species per unit area. For convenience in comparison, all densities were expressed as number of fish per hectare.

Statistical analysis

For each species and turbidity level, a frequency distribution and box plot of sighting distances were visually inspected for agreement with assumptions of the strip transect approach typically used in underwater observation studies (i.e., that sighting probability is 1.0 out to the limits of observer visibility). Although no formal statistical tests were performed, gross violations of the equal sighting probability assumption should be apparent using this exploratory approach.

Kendall's tau was used to test for correlation, and paired *t*-tests were used to test for significant differences between density estimates for each species and estimation method. The functional relationship between individual pairs of estimation techniques were estimated using Theil-Sen nonparametric regression (Hollander and Wolfe 1973).

Fig. 2. Box plots for Roanoke darter, Roanoke logperch, and black jumprock under low-turbidity (NTU < 3.5), moderate-turbidity (NTU ≥ 3.5 and NTU < 5.0), and high-turbidity (NTU > 5.0) conditions. The box encloses the middle 50% of the observations, the capped lines to the left and right of the box represent the 10 and 90% quantiles, respectively, and the squares to the left and right of the capped bars represent the 5 and 95% quantiles, respectively. Sample sizes are given in parentheses.



Results

Sighting distances

For all combinations of species and turbidity levels, there was evidence that the number of sightings decreased with increased distance of objects from the observer's line of travel. For the Roanoke darter, the number of observations increased slightly in the first 30 cm from the transect line and then declined out to point of maximum visibility at all turbidity levels (Fig. 1). Values for the median, 75, 90, and 95% quantiles for sighting distance all declined with increasing turbidity (Fig. 2). The 95% quantiles (equal to the boundary width for the Emlen estimates) under low-, moderate-, and high-turbidity conditions were 133, 104, and 89 cm, respectively.

For the Roanoke logperch under low-turbidity conditions, the number of observations remained relatively stable out to a distance of 75 cm and then began to decline (Fig. 1). Under moderate-turbidity levels, the number of sightings remained stable or increased slightly out to a distance of 100 cm and then began to decline. Under high-turbidity conditions, sightings increased out to 75 cm and then began to decline. Although values for the median, 75, 90, and 95% quantiles generally declined as turbidity increased, there were two exceptions (Fig. 2). The 95% quantile at low-turbidity levels was slightly lower than that at moderate-turbidity levels, and the 75% quantile at moderate turbidity levels was lower than that at high-turbidity levels. The 95% quantiles were 172, 184, and 142 cm for low-, moderate-, and high-turbidity levels, respectively. The high-turbidity results should be interpreted cautiously given the small sample size ($n = 27$).

For the black jumprock under low-turbidity conditions, there was a gradual increase in sightings out to a distance of 75 cm, followed by a decline (Fig. 1). Under moderate-turbidity conditions, sightings near the baseline were limited and there was a marked increase in the number of sightings out to approximately 75 cm, again followed by a decline in the number of sightings. At high-turbidity levels, sightings remained stable or increased only slightly out to 75 cm and then declined rapidly. As with the Roanoke logperch, the median and 90% quantile declined as turbidity increased (Fig. 2). The 75% quantile for sighting distance at moderate turbidity was slightly lower than at high turbidity, while the 95% quantile at low turbidity was slightly lower than at moderate turbidity. The 95% quantiles were 181, 188, and 121 cm for low-, moderate, and high-turbidity levels, respectively.

Estimator comparisons

For the Roanoke darter, the paired t-tests revealed significant differences between all but two pairs of estimation techniques. The Burnham estimates did not differ significantly from the Emlen estimates ($P > 0.05$), and the quadrat estimates did not differ significantly from the strip transect estimates ($P > 0.05$) (Table 1). The correlations between the quadrat and strip transect estimates were significant ($P < 0.05$), while correlations between all other pairs of estimates were highly significant ($P < 0.01$) (Table 1). Slopes of the Theil–Sen regressions indicated a one-to-one relationship between the Burnham and Emlen estimates, while the quadrat estimates were 66% lower and the strip transect estimates 73% lower than both the Burnham and Emlen estimates (Table 1). All of the Emlen estimates fell within the Burnham estimate 95% confidence intervals, while only five of the quadrat estimates and one of the strip transect estimates were contained within those same intervals (Table 2).

For the Roanoke logperch, paired t-tests showed that the strip transect estimates were significantly different from Burnham ($P = 0.004$), Emlen ($P = 0.007$), and quadrat ($P = 0.021$) estimates, while all other pairs of estimates

Table 1. Results of Theil–Sen regressions, correlations (Kendall's tau), and paired *t*-tests for comparisons among Burnham, Emlen, quadrat, and strip transect density estimates. For paired *t*-tests, the value is estimated mean difference between the first and second member of each pair. Significance levels for Kendall's tau and *t*-tests are given in parentheses. NS indicates $P > 0.05$.

Estimators compared	Regression equation	Kendall's tau	Estimated difference
	Roanoke darter		
Burnham–Emlen	Emlen = 0.99·Burnham + 32.93	0.956 (<0.001)	26.4 (NS)
Burnham–quadrat	Quadrat = 0.27·Burnham + 152.01	0.604 (0.003)	549.1 (0.007)
Burnham–strip	Strip = 0.34·Burnham − 25.09	0.692 (<0.001)	657.2 (<0.001)
Emlen–quadrat	Quadrat = 0.27·Emlen + 142.66	0.604 (0.003)	575.4 (0.005)
Emlen–strip	Strip = 0.34·Emlen − 31.79	0.692 (<0.001)	683.6 (<0.001)
Quadrat–strip	Strip = 0.59·Quadrat + 11.21	0.589 (0.019)	108.2 (NS)
	Roanoke logperch		
Burnham–Emlen	Emlen = 1.17·Burnham − 3.30	0.934 (<0.001)	5.7 (NS)
Burnham–quadrat	Quadrat = 0.61·Burnham + 13.19	0.508 (0.012)	9.1 (NS)
Burnham–strip	Strip = 0.39·Burnham + 3.42	0.780 (<0.001)	29.5 (0.004)
Emlen–quadrat	Quadrat = 0.45·Emlen + 19.20	0.508 (0.012)	14.8 (NS)
Emlen–strip	Strip = 0.33·Emlen + 5.70	0.758 (<0.001)	35.1 (0.007)
Quadrat–strip	Strip = 0.41·Quadrat + 3.81	0.464 (0.021)	20.4 (0.021)
	Black jumprock		
Burnham–Emlen	Emlen = 0.98·Burnham − 0.81	1.000 (<0.001)	4.3 (NS)
Burnham–quadrat	Quadrat = 0.75·Burnham + 46.25	0.487 (0.020)	57.7 (NS)
Burnham–strip	Strip = 0.38·Burnham + 14.56	0.590 (<0.005)	72.3 (0.004)
Emlen–quadrat	Quadrat = 0.77·Emlen + 40.19	0.487 (0.020)	62.1 (NS)
Emlen–strip	Strip = 0.33·Emlen + 5.70	0.590 (0.005)	68.0 (0.007)
Quadrat–strip	Strip = 0.41·Quadrat + 3.81	0.590 (0.005)	130.1 (0.025)

were not significantly different ($P > 0.05$) (Table 1). Correlations among the three underwater observation estimation methods were all highly significant ($P < 0.01$), while correlations between the underwater observation estimates and the electroshocking estimates were significant ($P < 0.05$) (Table 1). Slopes of the Theil–Sen regressions indicated that the strip transect estimates were 67, 60, and 60% lower than the Emlen, Burnham, and quadrat estimates, respectively, while the quadrat estimates were 40 and 55% lower than Burnham and Emlen estimates, respectively (Table 1). The slope of the Emlen–Burnham regression line indicated that the Emlen estimates were 17% higher than the Burnham estimates (Table 1). As with the Roanoke darter estimates, all of the Roanoke logperch Emlen estimates fell within the 95% confidence intervals for the Burnham estimates (Table 2). Only 10 of 14 quadrat estimates and 7 of 14 strip transect estimates fell within the Burnham confidence intervals.

The paired *t*-tests showed significant differences between the strip transect estimates and the Burnham ($P = 0.004$), Emlen ($P = 0.007$), and quadrat ($P = 0.025$) estimates for the black jumprock, while all other pairs of comparisons were nonsignificant ($P > 0.05$) (Table 1). Kendall's correlation coefficients indicated highly significant ($P < 0.01$) correlations between the Burnham and Emlen estimates, between the Burnham and strip transect estimates, between the Emlen and strip transect estimates, and between the strip transect and quadrat estimates (Table 1). The correlations between the quadrat estimates and the Burnham and Emlen estimates were significant ($P < 0.05$) (Table 1). Slopes of the Theil–Sen regressions indicated that the Burnham and Emlen estimates were equivalent (Table 1). The quadrat estimates were 25% lower than the Burnham estimates and 33% lower than the Emlen estimates, while the strip transect estimates were 77, 62, and 59% lower than the Emlen, Burnham, and quadrat estimates, respectively, as indicated by the Theil–Sen slopes (Table 1). The Emlen estimates were contained within the Burnham estimate 95% confidence intervals for all 13 sites, while the strip transect and quadrat estimates were within those same intervals at only 7 of the 13 sites (Table 2).

Discussion

Adequacy of the strip transect sighting model

The frequency distributions for the perpendicular sighting distance datasets indicate that distance sampling models that assume decreased sighting probability with increased distance of the target from the line of travel provide a more appropriate conceptual approach for abundance estimation of benthic riffle fishes than strip transect methods. For the Roanoke darter and Roanoke logperch, the frequency of sightings generally declined as a function of increased sighting distance. For the black jumprock, the general pattern was an initial increase in frequency of sightings, followed by a decline as distance from the line

Table 2. Abundance estimates (no./ha) for Roanoke darter, Roanoke logperch, and black jumprock from South Fork Roanoke River sites sampled in 1992 (SF1–SF9) and Roanoke River sites sampled in 1991 (RR1–RR5). For the Burnham estimates, 95% confidence intervals are given in parentheses.

Site	Burnham	Emlen	Strip transect	Quadrat
		Roanoke darter		
SF1	2359.9 (1845.1–3002.5)	2430.0	760.0	700.0
SF2	574.7 (405.3–814.7)	566.7	97.0	263.0
SF3	276.0 (180.1–423.1)	306.2	47.6	306.0
SF4	571.7 (396.4–824.5)	645.1	121.9	388.0
SF5	262.9 (151.3–457.1)	244.9	48.7	28.0
SF6	98.9 (50.4–194.2)	96.4	24.3	63.0
SF7	78.9 (41.6–149.4)	92.3	23.1	100.0
SF8	415.5 (259.0–666.5)	501.5	87.3	464.0
SF9	719.8 (457.2–1133.3)	798.5	81.6	417.0
RR1	1770.1 (1144.1–2738.7)	1725.9	660.1	1308.0
RR2	2313.8 (1511.6–3541.7)	2154.8	1164.0	650.0
RR3	1651.0 (1097.8–2483.1)	1609.8	759.1	550.0
RR4	1739.5 (1194.9–2532.4)	1929.6	370.4	230.0
RR5	914.0 (598.8–1466.2)	1013.9	835.7	593.0
		Roanoke logperch		
SF1	114.2 (7.8–75.9)	127.5	63.8	125.0
SF2	24.2 (66.2–196.9)	23.4	6.0	65.8
SF3	61.8 (30.9–123.9)	72.6	19.3	69.4
SF4	163.7 (94.1–284.7)	192.3	62.0	62.5
SF5	16.4 (3.7–72.9)	15.9	5.7	41.7
SF6	3.4 (0.4–26.4)	3.0	1.5	0.0
SF7	2.4 (0.3–19.0)	2.8	1.2	12.5
SF8	18.0 (5.1–63.9)	21.2	3.1	17.9
SF9	3.6 (0.5–28.2)	4.2	0.7	13.9
RR1	60.6 (28.2–130.1)	54.8	33.4	66.7
RR2	21.7 (10.5–44.8)	10.9	20.9	41.9
RR3	78.5 (46.0–133.9)	70.9	39.1	8.3
RR4	128.2 (79.3–207.1)	150.6	48.5	90.0
RR5	74.4 (42.9–129.4)	87.4	87.5	28.6
		Black jumprock		
SF1	66.3 (35.8–122.4)	86.1	52.1	178.0
SF2	109.3 (65.7–181.8)	99.4	35.0	92.1
SF3	241.9 (164.5–355.9)	237.7	74.8	305.6
SF4	252.9 (162.2–394.4)	248.5	95.0	125.0
SF5	24.0 (6.7–85.8)	21.8	7.6	13.9
SF6	7.9 (1.9–32.9)	7.2	4.6	25.0
SF7	4.9 (0.9–25.1)	4.8	2.4	62.5
SF8	190.9 (110.6–329.4)	187.5	33.0	89.3
SF9	260.5 (168.3–403.1)	261.3	54.0	222.2
RR1	271.8 (232.2–595.4)	335.7	302.5	583.3
RR2	122.5 (79.1–189.8)	111.4	108.0	758.3
RR3	140.3 (86.7–227.1)	126.7	140.3	75.0
RR4	— (—)	—	—	—
RR5	214.9 (151.4–305.1)	223.5	115.4	228.6

increased. If the basic assumption for a strip transect sighting model had been met, the frequency distributions should have been flat out to the maximum sighting distance, or at a minimum, for a significant portion of the total range of observations.

Further evidence that a strip transect sighting model is inappropriate for underwater estimation of benthic riffle fish density can be found in the box plots. The median value for sighting distance should be midway between the line of travel and maximum visibility distance. Making a conservative assumption that the 95% quantile for the sighting distance datasets equals the limit of visibility, then the median value is at approximately one third of the

95% quantile for each of the Roanoke darter datasets and at approximately two fifths of the 95% quantile for the Roanoke logperch and black jumprock datasets. Setting the limit of visibility equal to the Secchi disk extinction distance places the median value in the sighting datasets even further from the value expected under strip transect assumptions. The distribution of perpendicular sighting distances argues against the use of uncorrected counts as density estimates for benthic riffle fishes. Furthermore, the significant differences between the strip transect estimates and the Burnham and Emlen estimates show that setting a strip width equal to maximum visibility results in a strong negative bias in density estimates.

Adequacy of line transect models

Three basic assumptions underlie valid line transect estimation: (1) objects on the line of travel are detected with certainty, (2) objects are detected at their initial location, and (3) all measurements are exact (Buckland et al. 1993). Without ancillary data (i.e., an alternative manner of determining if fish on the line of travel are being missed), it is difficult to assess whether the first assumption is violated for the three species. Observations of fish behavior during the course of this study suggest that for the Roanoke logperch and black jumprock, this assumption is not violated. It is unlikely that a snorkeler could pass over an individual of either species without either seeing or disturbing (flushing) it. The interstitial spaces used by Roanoke logperch and black jumprock are usually not large enough to completely obscure an individual. Furthermore, when individuals of either species were observed on the centerline, some type of flight response was nearly always initiated before the snorkeler passed over them. A similar response was observed for the Roanoke darter. However, many substrate interstices were large enough to obscure the Roanoke darter, and some individuals may have taken cover and been overlooked. Density estimates for Roanoke darter may therefore be negatively biased, although the bias should be consistent across turbidity levels.

Gross violation of the second assumption (i.e., objects are detected at their original positions) results in frequency distributions with a peak in sightings well removed from the origin (Buckland et al. 1993). For the Roanoke logperch and Roanoke darter at all turbidity levels, there is only a slight increase in sightings for a short distance from the centerline (Fig. 1). For the black jumprock, there is a substantial increase in sightings at moderate turbidity levels (Fig. 1).

Behavior of the black jumprock provides a possible explanation for the observed pattern. Based on personal observations, black jumprock have a "comfort zone" of somewhat more than 2.0 m. Once a snorkeler moved within 2.0 m, many black jumprock exhibited a marked flight response. Under low-turbidity conditions, it was often possible to observe individuals or groups just before or immediately following initiation of the flight response. Under moderate turbidity conditions, many observations were of individuals already in motion. Black jumprock moving directly along the line of travel probably remained beyond the snorkeler's field of vision, while those moving at an angle to the line of travel may have been observed at some distance from the transect line. Such movement patterns would result in the observed moderate turbidity frequency distribution. At high-turbidity levels, observations were primarily of solitary individuals using crevices, boulders, and other substrate features as cover. Violation of the second assumption also results in a negative bias in density estimates (Buckland et al. 1993). Due to differences in black jumprock behavior under differing visibility conditions, the magnitude of the bias will not be consistent across visibility levels.

Given the manner in which data were collected in this study, the only likely source of error in measurement (assumption 3) would be if snorkelers moved off the transect line during the course of sampling. By moving off the transect line, an observer widens the area observed on one side of the line while narrowing it on the other. Although the distances between the line and any markers placed by the straying observer are still measured exactly, the perpendicular distances on the "wide side" are potentially beyond the observer's true field of vision and would result in inaccurate estimation of the sighting function. To minimize such violations, all personnel involved in data collection were fully informed of the importance of keeping the centerline of the body positioned over the transect line.

Line transect models are appropriate for estimation of Roanoke logperch densities because there were no apparent gross violations of the three assumptions. For the Roanoke darter, the assumption that all individuals on the centerline were seen with certainty may have been violated. Because this problem should remain constant across turbidity levels, at worst the estimates obtained using the line transect model should provide a consistent index of abundance for this species. Line transect sighting models may not be appropriate for species such as the black jumprock, whose behavioral response to divers violated at least two of the original assumptions.

Comparisons among estimates

All of the statistical tests indicate that the Emlen and Burnham models performed equally well in estimating abundance of the three species. The strong positive correlations between the visual estimates and the electroshocking estimates for all three species suggest that at a minimum, underwater observation provides estimates of relative abundance consistent at the ordinal level with the electroshocking technique. However, the quadrat estimates and the strip transect estimates were consistently lower than the Emlen and Burnham estimate across all three species. Wiley and Tsai (1983) estimated densities of a number of stream fishes using a backpack electroshocker and a three-pass depletion estimator. First-pass recoveries for tessellated darter (*Etheostoma olmstedi*) and white sucker (*Catostomus commersoni*), two species similar in size and behavior to the Roanoke darter and black jumprock, were 36 and 67% of their final density estimates, respectively. Because these values are very similar to the slopes of the quadrat – Burnham/Emlen regressions, underwater estimates for these two species would seem to be reasonably accurate. Unfortunately, because Wiley and Tsai (1983) did not sample a species with size and behavior similar to those of Roanoke logperch, no inferences can be drawn about the accuracy of the visual estimates for this species.

Conclusions

The simple Emlen model gave results similar to the more powerful Burnham model for all three species. Given the similarities in estimates, technique selection can be based on logistic consideration. With the Emlen model, once a correction factor is developed for a given species and environmental factor (i.e., turbidity level), it is unnecessary to obtain individual measurements on perpendicular distances. The only information needed to estimate abundance is the distance snorkeled, the number of fish seen, and the value of the environmental factor that determines the appropriate correction factor. The Burnham model requires information on the distribution of perpendicular sighting distances at each site for which an abundance estimate is needed. For fish biologists working with rare species, obtaining the number of observations needed for the Burnham model will often be impractical.

Once correction factors have been developed, the Emlen model lends itself well to studies designed to determine the distribution and abundance of benthic riffle fishes. To adequately assess fish populations, sampling should be extensive rather then intensive (Hankin and Reeves 1988; Matthews 1990), covering as many sites as possible. Based on our experience, it is possible to do two replicate passes on five to six 150-m riffle/run sequences in a single day. At that rate of sampling, reach and watershed level estimates of species distribution and abundance become possible.

Despite the positive aspects associated with the Emlen model, it is also important to note its limitations. As mentioned earlier, conceptually simple sighting models achieve their simplicity at a cost of restrictive assumptions about the shape of the sighting function. The similarity between the Emlen and Burnham estimates may be a fortuitous agreement between the two sets of sighting functions for the species and sighting conditions we encountered rather than representative of a broader pattern common to all benthic riffle fishes. Until additional comparative studies of the relationship between competing sighting models are available, inferring that models with restrictive assumptions about sighting function form (e.g., the Emlen model) will perform as well as the more flexible models (e.g., the Burnham model) is ill-advised. Furthermore, the lack of statistical techniques to estimate the variance of estimates obtained with the simpler sighting models could be a serious shortcoming, particularly if no other estimation techniques are used which provide estimates of the precision of abundance estimates.

The larger class of distance sampling models discussed by Buckland et al. (1993) (of which the Burnham model is just one example) have proven to be both accurate and precise in situations where population densities were known with certainty and all assumptions were met (Burnham et al. 1980; Bergstedt and Anderson 1990). Thus, one approach for routine density estimation is to assume that estimates obtained with these models reflect true population densities and to use these estimates to "calibrate" estimates obtained with one of the conceptually simpler models. The simpler model could then be used to routinely estimate densities. This is essentially the approach of Hankin and Reeves (1988) where three-pass depletion estimates were used to calibrate underwater counts of salmonids in streams in the Pacific Northwest.

The two line transect models we used represent a subset of the techniques available to fish biologists interested in using underwater observation. Additional research will no doubt reveal other models appropriate for situations different from this study. For target species other than open-water forms (e.g., salmonids, cyprinids), we strongly recommend models that incorporate factors correcting the number of fish observed for the probability of sighting. We also urge additional comparative studies where alternative estimation techniques are used to assess the precision and accuracy of underwater estimates.

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Time series bias in the estimation of density-dependent mortality in stock–recruitment models

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Abstract: Large biases can occur in parameter estimates for stock–recruitment models because the stock sizes are not chosen independently, being correlated with variability in recruitment. We examine the importance of this "time series bias" by a comprehensive analysis of available stock–recruitment data and the use of simulations. For semelparous species, i.e., species that reproduce only once, time series bias is important for all populations for which we had data. For iteroparous species, i.e., species that reproduce more than once, large biases occur if the populations are exploited at close to the maximum that is biologically possible. Notably, when there is autocorrelation in natural mortality, for univoltine species, the direction of bias is reversed due to model misspecification. Given moderate sample sizes and moderate levels of exploitation, time series bias is small for species such as Atlantic cod (*Gadus morhua*), for which α, the slope of the relationship between recruitment and number of spawners as the number of spawners goes to zero, is large. Time series bias will usually be important in species such as hakes (*Merluccius*) for which α appears to be relatively small.

Résumé : Des biais importants peuvent apparaître dans les calculs des paramètres utilisés dans les modèles stock–recrutement parce que la taille des stocks n'est pas choisie de façon indépendante mais corrélée à la variabilité du recrutement. Nous examinons l'importance de ce « biais des séries chronologiques » en pratiquant une analyse exhaustive des données existantes sur le rapport stock–recrutement et par le recours à des simulations. Pour les espèces sémelpares, c'est-à-dire celles qui ne se reproduisent qu'une fois, le biais des séries chronologiques est important pour toutes les populations sur lesquelles nous avions des données. Pour les espèces itéropares, c'est-à-dire celles qui se reproduisent plus d'une fois, des biais importants se manifestent si les populations sont exploitées près du maximum biologiquement possible. Lorsqu'il y a autocorrélation dans la mortalité naturelle, pour les espèces univoltines, on observe notamment que la direction du biais est inversée à cause d'erreurs de spécification du modèle. Étant donné que les tailles des échantillons et les niveaux d'exploitation sont modérés, le biais des séries chronologiques est faible pour des espèces comme la morue franche (*Gadus morhua*), pour laquelle α, la pente de la relation entre le recrutement et le nombre de géniteurs lorsque ce nombre approche de zéro, est élevé. Le biais des séries chronologiques est généralement important pour des espèces comme les merlus (*Merluccius*), pour lesquelles α semble relativement faible.

[Traduit par la Rédaction]

Introduction

The estimation of density-dependent mortality in fish, or in any species, is not a simple matter. Biases in the estimation of density-dependent processes have two widely known causes (Hilborn and Walters 1992). One cause is errors in the estimation of spawning population size, which tend to bias downward the estimation of the slope between recruitment and stock. This is well known as the errors in variables regression problem (Judge et al. 1984) and will not be considered here. Bias can also be introduced because the "independent" variable, spawning population size, is not independent of the interannual variation in the stock–recruitment relationship: for a given spawning population, above-average recruitment tends to result in higher spawning populations, while below-average recruitment tends to result in lower spawning populations. This is called *time series bias* and causes the density-dependent mortality to be overestimated. Walters (1985) was the first to identify this potentially important source of bias and has proposed a clever method of reducing the bias (Walters 1990). The time series bias is seldom dealt with when density dependence is examined, even though it has important theoretical and management implications.

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The purpose of this paper is to investigate when time series bias is important for the Ricker stock–recruitment model. We estimate bias using simulations with realistic parameter values obtained from a comprehensive analysis of stock and recruitment data (Myers et al. 1990, 1995; Myers and Cadigan 1993*a*).

Stock-recruitment model

We consider a Ricker stock–recruitment model that gives the number of recruits at age a in year $y + a$, R_{y+a}, resulting from the number of spawners in year y, S_y. The model has the form

$$[1] \qquad R_{y+a} = \alpha_0 S_y e^{-\beta S_y - \gamma},$$

where α_0 is the number of eggs produced per spawner, γ is the density-independent mortality that occurs after density-dependent population regulation, and β is the density-dependent mortality parameter.

We introduce variability in density-independent mortality *after* density-dependent population regulation. Therefore, we replace γ with $\gamma + \epsilon$, where ϵ is a normal random variable with mean 0 and variance σ_ϵ^2, giving

$$[2] \qquad R_{y+a} = \alpha_0 S_y e^{-\beta S_y - \gamma - \epsilon}.$$

The parameters α_0 and γ are not estimated separately; usually the slope at the origin, $\tilde{\alpha} = \alpha_0 e^{-\gamma}$, is estimated. Thus, our stock–recruitment model is

$$[3] \qquad R_{y+a} = \tilde{\alpha} S_y e^{-\beta S_y - \epsilon}.$$

We have expressed the equations as above to emphasize the exact assumptions that are usually implicitly made. This type of model may not be relevant to many populations in which density-dependent population regulation occurs *after* variable density-independent mortality (Myers and Cadigan 1993*a*, 1993*b*). A consideration of time series bias for such cases is beyond the scope of this paper.

Our model implies a lognormal distribution for the variability in recruitment for a given stock size. We made this assumption to make our results comparable with previous work. In Myers et al. (1995), we also estimate model parameters using a gamma distributional assumption. In general, the lognormal assumption can result in biases if the assumption is not exactly met (Myers and Pepin 1990). Also, Firth (1988) found that the loss in asymptotic efficiency of gamma maximum likelihood estimates when errors are lognormally distributed is less than the loss associated with lognormal maximum likelihood estimates when the errors are gamma distributed.

Realistic ranges of simulation parameters

In order to conduct plausible simulations to investigate the bias, realistic ranges of parameters were required. These were obtained from the database compiled by Myers et al. (1995) and included estimates of (1) a standardized form of the initial slope, (2) the density-dependent parameter, (3) the variance and autocorrelation in the stochastic component of natural mortality, and (4) the mean, variance, and autocorrelation in fishing mortality.

We used only data in which aging is not known to be problematic. In some species (e.g., tuna and swordfish), aging can only be undertaken via length-based methods; such stocks are not included in the analysis. The database contained 131 stocks having at least 15 years of data including 95 stocks having at least 20 years of data. However, not all data were available for all stocks.

Standardized initial slope

Estimates of the slope of the Ricker stock–recruitment curve at the origin were obtained from Myers et al. (1995). These slopes were converted into a *standardized* form which allows them to be compared across different stocks and species. The standardization is required because the model of Myers et al. (1995) differs from ours in two ways. First, they modelled *mean* recruitment, and second, for many stocks they used *spawning stock biomass* rather than number of spawners. A scaling factor is required for each of these differences.

The model used by Myers et al. (1995) can be written

$$[4] \qquad E(R_{y+a}) = \alpha^* B_y e^{-\beta B_y},$$

where α^* is the slope at the origin measured as recruits per unit biomass and B_y is the spawning stock biomass in year y. Our model, given in [3], can be rewritten in terms of B_y as

$$[5] \qquad R_{y+a} = \alpha^\dagger B_y e^{-\beta B_y - \epsilon},$$

where $\alpha^\dagger$ is the slope at the origin measured as recruits per unit biomass. To obtain the first scaling factor, note that the expected recruitment for our model is

$$[6] \qquad E(R_{y+a}) = (\alpha^\dagger e^{\frac{1}{2}\sigma_\epsilon^2}) B_y e^{-\beta B_y},$$

since $E(e^{-\epsilon}) = e^{\frac{1}{2}\sigma_\epsilon^2}$. Comparing [4] and [6], we see that

$$[7] \qquad \alpha^* = \alpha^\dagger e^{\frac{1}{2}\sigma_\epsilon^2}.$$

That is, estimates from Myers et al. (1995) of the slope at the origin will be inflated by a factor $e^{\frac{1}{2}\sigma_\epsilon^2}$.

To obtain the second scaling factor, we being by defining the spawning stock biomass in year y as

$$[8] \qquad B_y = \sum_{c=1}^{A} R_{y-c} w_{y,a+c} P_{y,a+c} e^{-\sum_{b=1}^{c-1} (M_{y-c+b,a+b} + F_{y-c+b,a+b})},$$

where A is the maximum age observed, R_{y-c} is the recruitment in year $y - c$ (at age a), $w_{y,a+c}$ is the weight at age $a + c$ at the beginning of year y, $P_{y,a+c}$ is the proportion mature at age $a + c$ in year y, $M_{y-c+b,a+b}$ is natural mortality at age $a + b$ in year $y - c + b$, and $F_{y-c+b,a+b}$ is the fishing mortality at age $a + b$ in year $y - c + b$. The scaling factor we use is $\mathrm{SPR}_{F=0}$, the spawner quantity per recruit when fishing mortality is zero (Goodyear 1977; Gabriel et al. 1989; Mace 1994). This scaling factor may be used in general to obtain the same units for spawner quantity and recruitment. In the present case the spawner quantity is expressed as spawning stock biomass. Consider a year-class of R individuals. Over the lifetime of the year-class, the total contribution to the spawning stock biomass is

Fig. 1. Estimates of the initial slope, α, of the Ricker stock–recruitment model for (*a*) semelparous species and (*b*) iteroparous species (from Myers et al. 1995). In Fig. 1*b*, estimates greater than or equal to 70 have been collected at 70.

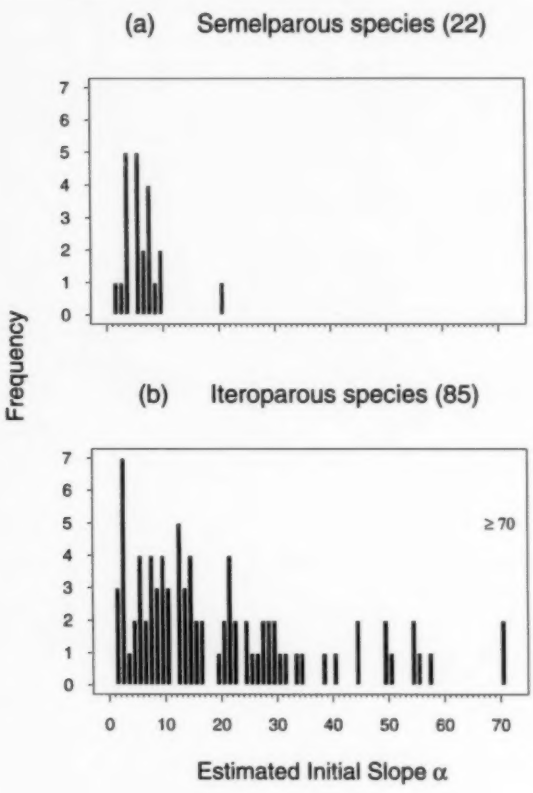
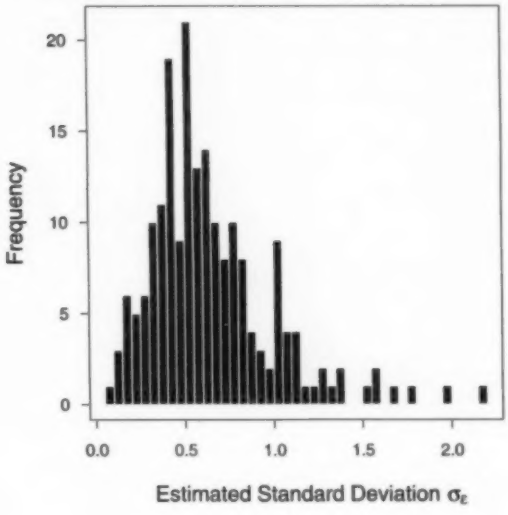


Fig. 2. Estimates of σ_ε, the standard deviation of log residuals from a Ricker stock–recruitment model, for 169 populations (from Myers et al. 1995).



$$[9] \quad \text{Total}(B) = \sum_{i=1}^{A} w_i P_i R e^{-\sum_{c=1}^{i-1} M_c + F_c},$$

where w_i is the weight at age i, P_i is the proportion mature at age i, M_c is the natural mortality at age c, and F_c is the fishing mortality at age c. Setting $F_c = 0$ and dividing by R gives

$$[10] \quad \text{SPR}_{F=0} = \sum_{i=1}^{A} w_i P_i e^{-\sum_{c=1}^{i-1} M_c}.$$

The *standardized* initial slope, α, is obtained by scaling the initial slope $\alpha^\dagger$ by $\text{SPR}_{F=0}$, i.e.,

$$[11] \quad \alpha = \alpha^\dagger \cdot \text{SPR}_{F=0} = \alpha^\dagger e^{-\frac{1}{2}\sigma_\varepsilon^2} \text{SPR}_{F=0}.$$

Estimates of $\text{SPR}_{F=0}$ were extracted from the database compiled by Mace and Sissenwine (1993) and are based on the same age-at-recruitment, growth, and maturation schedules used in the Myers et al. (1995) database of stock and recruitment estimates.

Applying these scaling factors, we obtained 107 estimates of α. We will consider separately semelparous species, i.e., species that reproduce only once, and iteroparous species, i.e., species that survive after reproduction.

There were 22 estimates of α for semelparous species (Fig. 1*a*). These were for the Pacific salmon (*Oncorhynchus*). The median estimated α is 5.8; this implies that at low levels of spawners, a spawner should produce about six replacement spawners in the absence of fishing mortality. There were 85 estimates of α for iteroparous species (Fig. 1*b*). The distribution is positively skewed with a median of 14.4. We investigate five representative values of α: 2, 4, 6, 10, and 30.

Density-dependent parameter

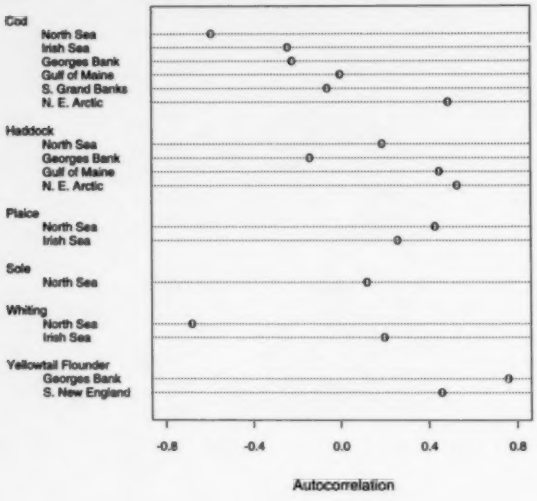
The parameter β varies with the size of the population and cannot readily be compared between populations. Fortunately, time series bias is relatively insensitive to β (Walters 1985). We initially assume β = 1, where the units of β are one over the units of the spawners.

Stochastic component of natural mortality

The standard deviation in the interannual variability in mortality, σ_ε, is available from Myers et al. (1995) for 169 populations using the Ricker model under the assumption that the variation follows a lognormal distribution (Fig. 2). The median is 0.57. We chose σ_ε of 0.2, 0.5, and 1.0 to investigate in the simulations, which represent the 5th, 36th, and 85th percentiles, respectively.

Walters (1990) showed that autocorrelation in the stochastic component of natural mortality increased time series bias; however, estimation of this autocorrelation is not easy. First, the simple sample autocorrelation is negatively biased (Kendall et al. 1983, section 48.3). Second, if research vessel estimates of abundance at age are used, estimation error will bias the sample autocorrelation towards zero. Third, aging errors in the analysis of commercial

Fig. 3. First-order autocorrelations in log recruitment estimated from multiple research surveys using a latent variable model (from Myers and Cadigan 1993*a*).

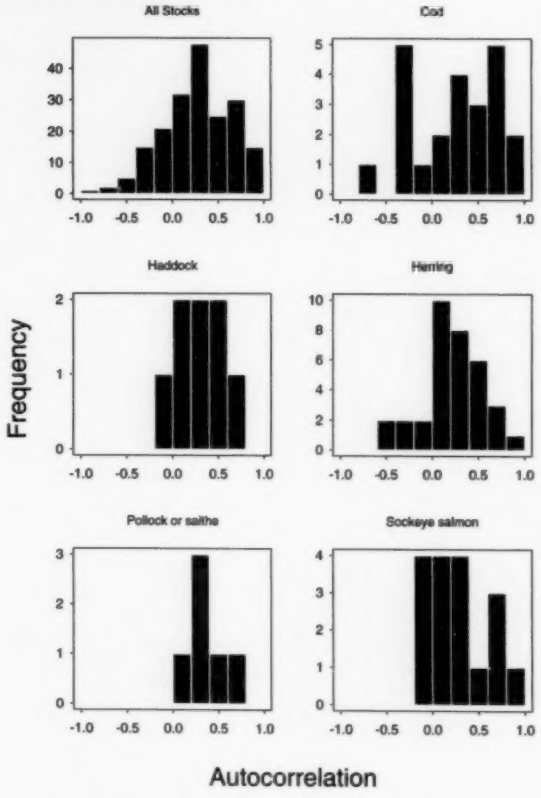


catch-at-age data will make recruitment estimates appear to be positively correlated, even if they are not (Bradford 1991).

Each of these difficulties was overcome by Myers and Cadigan (1993*a*) using a latent variable model of multiple research survey estimates of abundance to estimate the autocorrelation between adjacent cohorts (Fig. 3). Their method is not biased by aging errors because they used only data from juveniles where the aging errors are minimal. They also found that other types of biases in the estimates of autocorrelations were small. They found evidence of negative autocorrelation between adjacent cohorts of cod; this pattern is consistent with density-dependent mortality between adjacent cohorts (Fig. 3). There is one region where positive autocorrelation does appear to be real for cod; in the Northeast Arctic, both cod and haddock appear to have significantly positive autocorrelation. This is probably because the autocorrelation in the environment is more important in this region. The autocorrelations are positive for all the flatfish populations examined; however, even here the median, 0.43, is not large. It appears that autocorrelation is typically small, and even negative if there is density-dependent mortality between adjacent cohorts. There may be populations where the autocorrelation is greater, but probably not much greater than 0.5. It is apparent that the degree of autocorrelation estimated by Myers and Cadigan (1993*a*) is very much lower than that estimated by commercial catch-at-age data. This is almost certainly caused by the aging error used in the commercial catch-at-age analysis.

For completeness, we examine the autocorrelation in the log-transformed residuals from the Ricker stock–recruitment model. The autocorrelation estimated from VPA's is generally positive (Fig. 4). This includes almost all the data in Fig. 4 with the exception of the data on salmonids. Note that the only iteroparous species where we have several

Fig. 4. First-order autocorrelations in log residuals from a Ricker stock–recruitment model for 169 populations (from Myers et al. 1995).



data sets, sockeye salmon (*Oncorhynchus nerka*), has median autocorrelation of 0.26. For the simulations, we usually investigate autocorrelation, ρ, of 0 and 0.5.

Fishing mortality

In order to provide realistic estimates of the statistical properties of fishing mortality, we examined the estimates of fishing mortality compiled in Myers et al. (1995). We analyzed those data series having at least 20 years of data, for a total of 95 series (Fig. 5). The 10th percentile, median, and 90th percentile mean fishing mortalities are 0.25, 0.55, and 1.28, respectively. The median standard deviation in fishing mortality is 0.21. The median autocorrelation in fishing mortality is 0.47. Our simulations begin with mean fishing mortality $\mu_F = 0.5$, standard deviation $\sigma_F = 0.18$ (based on an earlier estimate), and autocorrelation $\rho_F = 0.5$.

Simulations to estimate bias

Populations were simulated using combinations of the parameter values described in the previous section. For each combination of parameters, 500 simulations were used. We investigated sample sizes of 10, 15, and 30. Sample sizes of 10 were investigated for completeness; we do not recommend such small sample sizes in general.

Fishing mortality

For semelparous species, fishing mortality is assumed to occur only on fish before they spawn. In this case the dynamics of the spawners is given by

$$[12] \quad S_y = e^{-F_y} R_y.$$

Combining the stock–recruitment model [3] with [12] gives

$$[13] \quad S_{y+a} = e^{-F_{y+a}} \tilde{\alpha} S_y e^{-\beta S_y - \epsilon}.$$

In the semelparous case, spawners and recruitment have the same units; therefore, $\tilde{\alpha}$ is equal to the standardized initial slope, α; thus

$$[14] \quad S_{y+a} = e^{-F_{y+a}} \alpha S_y e^{-\beta S_y - \epsilon}.$$

Note that the product $e^{-F_{y+a}}\,\alpha$ gives the initial slope relative to the intensity of fishing. If fishing mortality is constant at F_0, then a deterministic equilibrium is given by

$$[15] \quad S_0 = \frac{1}{\beta} \ln\left(\frac{\alpha}{e^{F_0}}\right).$$

In most simulations of fish populations, fishing mortality is held constant; however, it is probably best considered in the context of time series analysis (Gudmundsson 1987, 1994). We simulate the interannual variation in fishing mortality by a Markov process, i.e., a first-order autoregressive model. To prevent the process from straying beyond realistic values, we used reflecting boundaries at 0.1 and 1.5. Let $\dot{F}_y$ represent the deviation in year y of fishing mortality from the long-term mean, i.e., $\dot{F}_y = F_y - \mu_F$, where μ_F is the long-term mean. We assume that fully recruited fishing mortality is described by

$$[16] \quad \dot{F}_y = \rho_F \dot{F}_{y-1} + \zeta_y,$$

where ρ_F is the autocorrelation between adjacent fishing mortalities and the $\{\zeta_y\}$ are a sequence of independent and identically distributed normal random variables with mean zero and variance σ_ζ^2. Therefore

$$[17] \quad \mathrm{Var}(F_y) = \sigma_F^2 = \frac{\sigma_\zeta^2}{1-\rho_F^2}.$$

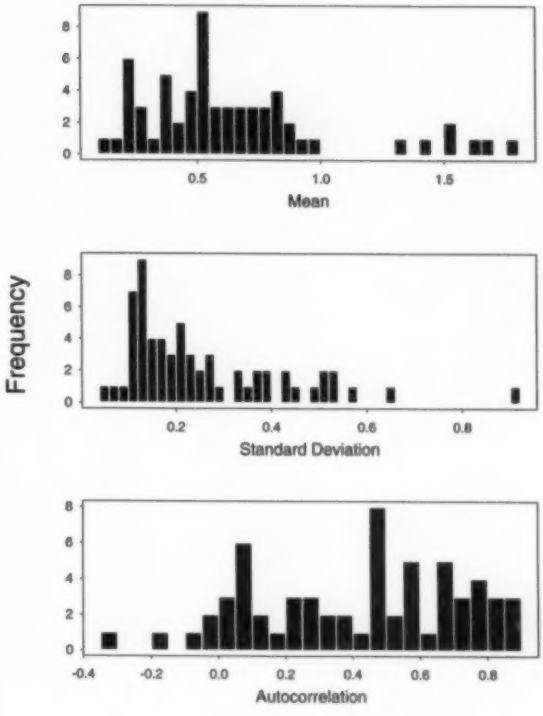
We initialized the simulations by setting the fishing mortality equal to its mean and calculating the resulting equilibrium spawners using [15]. To ensure that the initial conditions were not too important for the results, we simulated an initial 10 years of data that were not used in the bias estimates.

Modelling age structure

To investigate the effect of age structure, we use the Clark (1976) model, which avoids the complicated formalism of full age-structured models. Let the survival of adults between years in the absence of fishing mortality be $\ell = e^{-M}$. The formulation we use simulates a fishery that occurs on the spawning ground just before spawning occurs. The number of spawners in year y for our iteroparous model is given by

$$[18] \quad S_y = e^{-F_y}(\ell S_{y-1} + R_y \mathrm{SPR}_{F=0}),$$

Fig. 5. Mean, standard deviation, and first-order autocorrelation in fishing mortality among years for 57 populations (from Myers et al. 1995).



where, in this case, $\mathrm{SPR}_{F=0}$ is the *number* of spawners per recruit at zero fishing mortality. Recall that in the semelparous case, in the absence of fishing mortality, a single recruit results in a single spawner; hence, $\mathrm{SPR}_{F=0} = 1$, i.e., $\alpha = \tilde{\alpha}$. In the iteroparous case, however,

$$[19] \quad \mathrm{SPR}_{F=0} = \sum_{i=0}^{\infty} \ell^i = \frac{1}{1-\ell}.$$

Thus the standardized initial slope is given by

$$[20] \quad \alpha = \tilde{\alpha} \cdot \mathrm{SPR}_{F=0} = \frac{\tilde{\alpha}}{1-\ell},$$

i.e., $\tilde{\alpha} = \alpha(1 - \ell)$. Combining the stock–recruitment model [3] with [18] and [20] gives

$$[21] \quad S_{y+a} = e^{-F_{y+a}}(\ell S_{y+a-1} + \alpha S_y e^{-\beta S_y - \epsilon}).$$

If fishing mortality is constant at F_0, then a deterministic equilibrium is given by

$$[22] \quad S_0 = \frac{1}{\beta} \ln\left(\frac{\alpha}{e^{F_0} - \ell}\right).$$

As in the semelparous case, simulated recruitments were generated using [3] but with $\tilde{\alpha} = \alpha(1 - \ell)$. We allowed fishing mortality to vary in the same manner as we did for the semelparous case, i.e., as an autoregressive process.

Table 1. Percent bias in estimates of the β parameter of the Ricker stock–recruitment model from simulations of a semelparous population with an age-of-maturity of 4 years. The bias is given for selected values of the following parameters: variability in the stochastic component of natural mortality, σ_ϵ; sample size, n; autocorrelation in the stochastic component of natural mortality, ρ; and slope at the origin; α. Fishing mortality was assumed to be an autoregressive process with mean $\mu_F = 0.5$, standard deviation 0.18, and autocorrelation 0.5. The term $\alpha e^{-\mu_F} = \alpha e^{-0.5}$ is given in parentheses next to α. The percent bias has been rounded to the nearest integer.

			α ($\alpha e^{-\mu_F} = \alpha e^{-0.5}$)			
σ_ϵ	n	ρ	2 (1.21)	4 (2.43)	6 (3.64)	10 (6.06)
0.2	10	0	45	5	1	−1
		0.5	75	12	6	1
	15	0	38	4	1	0
		0.5	60	10	4	0
	30	0	29	2	0	−1
		0.5	40	2	1	−1
0.5	10	0	66	8	2	0
		0.5	117	19	9	2
	15	0	50	5	2	0
		0.5	101	17	4	0
	30	0	41	4	1	−1
		0.5	71	4	0	−1
1	10	0	72	7	4	2
		0.5	122	18	7	2
	15	0	61	5	0	1
		0.5	120	12	5	1
	30	0	32	3	0	0
		0.5	69	4	1	0

Estimation

Standard linear least squares estimation was used to estimate the parameters of the Ricker stock–recruitment model. The regression equation is obtained from [3] by dividing both sides by S_y and taking the logarithm:

$$[23] \quad \log\left(\frac{R_{y+a}}{S_y}\right) = \log(\tilde{\alpha}) - \beta S_y - \epsilon.$$

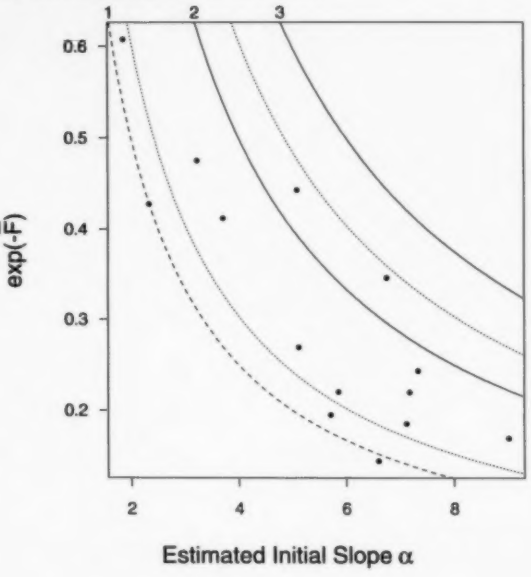
We estimated the mean and standard error of the estimates of the density-dependent mortality term, β. We also estimated the mean and standard error of the residual variance.

Results and discussion

Semelparous species

We first consider the bias in the Ricker stock–recruitment model for species with semelparous reproduction using the base fishing mortality assumptions described above (Table 1). We use an age at maturity of 4 years, which simulates a sockeye salmon life history. The time series bias does not appear to be important unless $\alpha e^{-\mu_F}$, the product of the standardized initial slope, α, and the survival after harvest at mean fishing mortality, $e^{-\mu_F}$, is small. Kope (1988) noted that changes in the survival rate have the same effect on bias as changes in α. The bias is increased for smaller sample sizes, increased recruitment variation, and autocorrelated recruitment, but these factors are of secondary importance. Thus, our conclusions about time series bias for semelparous species will primarily depend on α and harvest rates for real populations.

Fig. 6. Survival at mean fishing mortality, $e^{-\bar{F}}$, versus estimated initial slope α for semelparous populations. The time series bias is related to $\alpha e^{-\bar{F}}$, several values of which are given by the contour lines. The contour lines are labelled at the top of the figure.



We have plotted the survival at mean fishing mortality, $\bar{F}$, and estimated α for every semelparous population for which data on both parameters were available (Fig. 6). All population parameters fall within the range where bias is important. Note that there is a very strong relationship between the estimated α and $e^{-\bar{F}}$. Most populations in Fig. 6 lie close to the curve $\alpha e^{-\bar{F}} = 1$. We interpret this to mean that fishing mortality is maintained at close to a biological maximum possible for these populations.

A fundamental difficulty is apparent in Fig. 6: a small change in the survival rate or in the estimate of α can lead to very large changes in the bias. Since both survival rate and α are estimated with error, it is inherently difficult to correct for the bias. The bias correction factor may vary by an order of magnitude within the confidence region for these parameters alone. This is probably why previous attempts to estimate the bias correction (e.g., Cass 1989), have led to order of magnitude differences in the bias correction among populations of sockeye salmon within the Fraser River system.

Our results are consistent with those of Walters (1985, 1990), Caputi (1988), and Kope (1988). However, the

Table 2. Percent bias in estimates of the β parameter of the Ricker stock–recruitment model from simulations of an iteroparous population with an adult natural survival of 0.8 and an age-of-maturity of 3 years. The bias is given for selected values of the following parameters: variability in the stochastic component of natural mortality, σ_ϵ; sample size, n; autocorrelation in the stochastic component of natural mortality, ρ; and standardized slope at the origin; α. Parameter combinations for which there were no equilibrium numbers of spawners are denoted by a dash. Fishing mortality was assumed to be an autoregressive process with mean 0.5, standard deviation 0.18, and autocorrelation 0.5. The percent bias has been rounded to the nearest integer.

			α			
σ_ϵ	n	ρ	4	6	10	30
0.2	10	0	—	18	6	−1
		0.5	—	38	8	0
	15	0	—	12	4	0
		0.5	—	33	6	0
	30	0	—	9	3	0
		0.5	—	19	3	−1
0.5	10	0	—	65	10	1
		0.5	—	97	26	3
	15	0	—	55	9	1
		0.5	—	93	21	0
	30	0	—	33	6	0
		0.5	—	44	5	−3
1	10	0	—	63	14	2
		0.5	—	158	36	6
	15	0	—	47	11	0
		0.5	—	92	27	2
	30	0	—	22	5	0
		0.5	—	45	5	−3

Table 3. Percent bias in estimates of the β parameter of the Ricker stock–recruitment model from simulations of an iteroparous population. The sample size, n, is fixed at 15. The bias is given for selected values of the following parameters: age of maturity, a_{mat}; survival of adults between years in the absence of fishing mortality, ℓ; mean fishing mortality, μ_F; and standardized slope at the origin, α. Fishing mortality was assumed to be an autoregressive process with standard deviation 0.18 and autocorrelation 0.5. Parameter combinations for which there were no equilibrium numbers of spawners are denoted by a dash. The percent bias has been rounded to the nearest integer.

			α		
a_{mat}	ℓ	μ_F	6	10	30
1	0.5	0.5	14	6	−1
		0.8	32	11	−1
	0.8	0.5	79	25	5
		0.8	—	82	8
	0.9	0.5	—	98	14
		0.8	—	—	25
3	0.5	0.5	7	1	−1
		0.8	23	5	−1
	0.8	0.5	48	11	0
		0.8	—	43	3
	0.9	0.5	—	60	3
		0.8	—	—	12
6	0.5	0.5	7	2	−1
		0.8	15	4	0
	0.8	0.5	32	7	0
		0.8	—	29	2
	0.9	0.5	—	32	3
		0.8	—	—	8

biases that they estimated are greater than those inferred for many of the populations for which we have data. This is because the α they used was smaller than that observed for most populations. For example, Walters (1985, 1990) and Kope (1988) assumed $\alpha = e \approx 2.72$; only two of the semelparous populations from Myers et al. (1995) had α's that low.

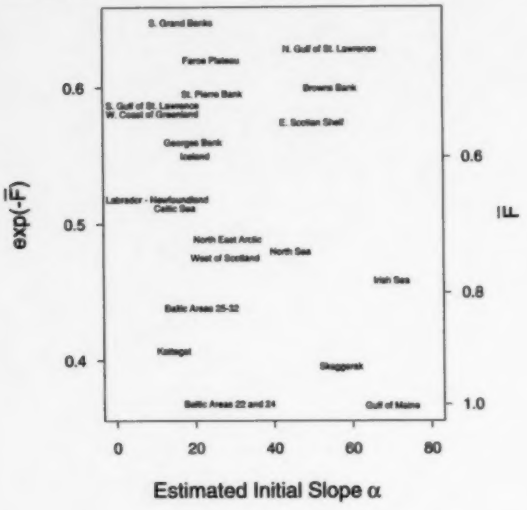
The most important factor in the consideration of time series bias for semelparous species is the product of the survival from the fishery and the reproduction rate as the number of spawners goes to zero, αe^{-F}. If this factor is around 2.5, then the bias in the estimate of the density-dependent mortality ranges from 2 to 19% (Table 1). The bias is greater when data series are short (around 10 data points) and have large stochastic variability.

The biases in the estimates of α are also positive, but are typically approximately half those of β. Thus, the estimated α in Fig. 6 will be positively biased, and consequently the bias in estimates of β will be even greater than that estimated.

Iteroparous species

Our initial model (Table 1) of a semelparous species was modified to describe an iteroparous species with an age of maturity of 3 years and an adult natural survival of 0.8 (Table 2). Time series bias appears to be more important with overlapping generations if similar values of α are assumed; however, species with survival after reproduction tend to have larger α's (Fig. 1). If α is around 6, time series bias is important under our base assumptions about fishing mortality. For an α of 10 with the assumed fishing mortality, time series bias reduces to what may be an

Fig. 7. Survival at mean fishing mortality, $e^{-\bar{F}}$, versus estimated initial slope α for cod populations. A label for each population is centred at the estimated values. For reference, the right-hand axis gives values of the mean fishing mortality, $\bar{F}$.



acceptable level of approximately 20%. Autocorrelated stochasticity in recruitment increases the bias.

We investigated the effect of changing the age at maturity, α_{mat}, natural survival after reproduction, ℓ, and mean fishing mortality, μ_F (Table 3). If natural survival after reproduction decreases, then the bias decreases. This is consistent with the results from Table 1 where the survival was zero. If age of maturity decreases, the bias increases slightly.

Among iteroparous species, there are some species for which time series bias for the Ricker model will be important. It is worth examining these species groups in more detail. The hakes (*Merluccius*) appear to have a relatively low α; this would suggest that time series bias will be important. The α for *Merluccius* ranges from 2.6 to 13.0 (mean = 5.87, n = 6). However, the natural mortality for this genus is usually assumed to be high (M = 0.4), which would substantially reduce the bias (Table 3). The same is true of the California sardine (*Sardinops caerulea*) for which natural mortality is also assumed to be 0.4 and the α is very low, 2.75. However, the fishing mortality for the sardine was unsustainable, so that large biases can be expected for the species.

Some species, such as Atlantic cod (*Gadus morhua*), appear to have such a large α that time series bias will generally not be important unless there is overfishing (Fig. 7). Given the importance of this species, we will examine it in more detail. Many of the cod populations have been subjected to high fishing mortality. There are three populations with α's around 10, cod in West Greenland, off Labrador, and in the southern Gulf of St. Lawrence. These species mature at approximately age 6, have a natural survival of approximately 0.8, and have been subjected to an average fishing mortality of between 0.55 and 0.7. Thus, the bias in β should be between 7 and 29% (Table 3). Similarly, cod in the Baltic Sea and the Kattegat have α's from approximately 15 to 30, mature around age 3, have a natural survival of approximately 0.8, and have experienced very high levels of exploitation. In these cases, the bias in β may be a problem.

Table 4. Percent bias in estimates of the β parameter of the Ricker stock–recruitment model from simulations of a semelparous population with an age-of-maturity of 1 year and with autocorrelation in the stochastic component of natural mortality, ρ = 0.5. The bias is given for selected values of the following parameters: variability in the stochastic component of natural mortality, σ_ϵ; sample size, n; and slope at the origin, α. Fishing mortality was assumed to be an autoregressive process with mean μ_F = 0.5, standard deviation 0.18, and autocorrelation 0.5. The percent bias has been rounded to the nearest integer.

		α			
σ_ϵ	n	4	6	10	30
0.2	10	−15	−12	−10	−1
	15	−18	−17	−11	−1
	30	−24	−19	−9	−1
0.5	10	−18	−19	−15	−3
	15	−27	−22	−14	−3
	30	−33	−25	−15	−2
1	10	−8	−14	−14	−6
	15	−17	−18	−13	−4
	30	−26	−19	−13	−4

Other species, such as herring (*Clupea*), are in between cod and the hakes; a few populations appear to have a low α, which may result in significant biases. There is one case for which bias is always a concern. If data are only available for a population during a time period when the fishing mortality was unsustainable, (i.e., one has a population going into commercial extinction), then there is a good chance that estimates for such a population may be significantly biased, and simulation tests should be carried out.

As in the semelparous case, the α's we estimated (Figs. 1 and 6) will be positively biased. Consequently the bias in β will be greater than we would expect.

We investigated the robustness of the above conclusions by considering alternative parameters. The general conclusions were not changed when β was reduced by two orders of magnitude. This conclusion holds for the semelparous simulations as well and is consistent with other studies (Walters 1985; Cass 1989).

Univoltine species

There is one case we investigated in which autocorrelated stochastic mortality does not create a positive bias (Table 4).

If age-at-maturity is 1, autocorrelated mortality causes the mortality to be underestimated in all cases. However, this bias is caused by model misspecification rather than time series bias; the density-dependent parameter is confounded by environmental autocorrelation. Nevertheless, time series bias can be discerned in Table 4; for small α, the estimates are less negative for small sample size.

Conclusions

The main result of the analysis is to identify the conditions under which time series bias for the Ricker model is important. For semelparous species (e.g., Pacific salmon) the situation is clear: time series bias will generally be very important and cannot be ignored (see also Korman et al. 1995).

For iteroparous species the situation is more complex. For species with low α's, such as hakes and sardines, time series bias can be assumed to be important. For species with generally high α's, such as cod, time series bias can sometimes be ignored for the Ricker model. Species such as herring must be examined on a case-by-case basis. The most outstanding observation from the simulations concerns the effects of overexploitation. Overexploited stocks result in high bias, i.e., if a population is overexploited relative to its life history, then large biases probably occur (Table 3).

A further important result from our analysis is that density-dependent mortality is significantly negatively biased for univoltine (one generation per year) species if environmental variability is positively autocorrelated. The direction of this bias is the opposite of that expected for time series bias. This is caused by confounding of the autocorrelated environmental stochasticity with density dependence with a 1-year lag. Although this result is not relevant for most fish populations, it does imply that the use of the Ricker model for insect populations (e.g., Turchin 1990; Turchin and Taylor 1992; Woiwod and Hanski 1992) may underestimate the importance of density-dependent mortality. This effect may be important for univoltine marine species, such as some copepods and squid.

For most marine populations, α appears to be much larger, which appears to allow them to sustain high commercial fishing mortality. Such a high potential population growth rate is impossible for many species (e.g., mammals). Time series bias may be important for such species.

While our results indicate that time series bias may not be important for some populations, they suggest that for all semelparous species and for iteroparous species being subjected to unsustainable levels of exploitation, time series bias is likely to be a problem. Certainly, any attempt to fit more complex models, e.g., three-parameter stock–recruitment models, should only be done in conjunction with extensive simulation models. We fully agree with Walters' (1985) suggestion that any estimation problem using time series should be checked for bias using simulation studies.

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General guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. In 1995 the *Journal* introduced a new internal design. Authors are requested to refer to an issue in the new style for details of layout, especially for tables and reference lists. General guidelines follow.

Type the manuscript on white paper (8.5 3 11 in.) on only one side of the page. Leave all margins at least 1 in. wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Use italic font if available; when not available, underline material that is to be set in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparison? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what readers need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

(1) Limit abstract to one paragraph of about 175 words.

(2) Double-space all elements of the manuscript, including references, table captions, and figure legends.

(3) Avoid exclusive use of capitals anywhere in the manuscript, including headings, table captions, and figure legends.

(4) Italicize or underline only Latin names of organisms and appropriate statistical and mathematical notations.

(5) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

(6) Avoid ambiguous forms such as g C/m^2/d; use g C·m^{-2}·d^{-1}.

(7) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

(8) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

(9) Include acknowledgements section at manuscript stage, not when page proofs arrive.

(10) Provide the person's initials and mailing address when referring to personal communications.

(11) Delete unnecessary references that do not apply directly to the problem.

(12) Check references carefully against test citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g., available from Department of Economics, Simon Fraser University, Burnaby, BC V5A 1S6, Canada.

(13) Delete commas between name and date in citations such as (Smith 1990) and do not underline "et al."

(14) Replace 10^n in table headings and figure axes with appropriate SI prefixes or use words (e.g., thousands).

(15) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints or laser printouts of figures, or originals if they do not exceed 8.5 × 11 in., on request for publication. Use the same type of lettering throughout if possible.

Parts of the manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g., X ray, chromatography, mathematical analysis). Titles should not begin

with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to..." or "Investigations on..." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers in preparing titles for keyword indexes.

Abstract

The abstract should succintly mirror the contents and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as "...is discussed" or "...was found"; be specific. As the abstract is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents and be completely self-contained (i.e., any *essential* references) in a retrievable form (e.g., R.B. Deriso. 1980. Can. J. Fish. Aquat. Sci. 37: 268–282).

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and methods

Materials and methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine...we..." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and condense them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the captions. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgements

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. **Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence.** Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit a 1-, 1½-, or 2-column width of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra space instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript lowercase letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed within the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions, laser printouts, or the original artwork (no larger than 8.5 × 11 in.) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures produced on laser printers are acceptable; figures produced on dot matrix printers are not acceptable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

CorelDraw! is our preferred graphics program, which can accommodate a variety of IBM-compatible formats (e.g., EPS, .TIF). Clearly label the disk with the program, version number, and the figure numbers with the corresponding filenames and extensions. Use the correct extension(s) for the program(s) used, or otherwise our system may not be able to read or open the files.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the understanding of the text. Extra costs for the reproduction of color plates are charged to the author; currently the cost is $1000 per page or part of a page. Further details may be obtained from the Editorial Office or the Publishing Department.

Submit only photographs of the highest quality for color reproduction. We prefer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Preparing Photographs and Illustrations for Reproductions, a pamphlet prepared by the Publishing Office, is available from the Editorial Office.

Mathematical expressions, equations, and formulae

Leave three spaces above and below lines that have much superscript material, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between lowercase *l* and the numeral *one*, and between capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetres per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	µm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar mass	M
mole	mol
moles per litre	mol/L, M
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne (metric ton)	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the...), or the alternative sequence month-day-year may be used with the year offset by commas (On October 9, 1983, the...).

Reference citations in text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared..." or "...were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the fist author followed by "et al." (not italicized)... "Brown et al. (1983) compared..." or "...were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "personal communication."

Unpublished data

If an unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)." Only those manuscripts that are in galley or page proof stage or for which there is an acceptance letter can be considered in press. If an article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpublished data" in the text and do not include it in the reference list.

Reference lists

References should be listed at the end of the paper in alphabetical order according to the surnames of the first author. References with the same first author are listed in the following order. (1) Papers with **one author only** are listed first in chronological order, beginning with the earliest paper. (2) Papers with **dual authorship** follow and are listed in alphabetical order by the last name of the second author. (3) Papers with **three or more authors** appear after the dual-authored papers and are arranged chronologically.

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *CASSI* or *Serial Sources for the BIOSIS Data Base*) for references.

Journal article
Peterman, R.M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal
Gordon, D.C., Jr., and A.S. Hourston [Ed]. 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 365 p.

Book in a series
Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series
LeBlond, P.H., and L.A. Mysak. 1978. Waves in the ocean. Elsevier, New York, N.Y. 602 p.

Part of book
Healey, M.C. 1980. The ecology of juvenile salmon in Georgia Strait, Britsh Columbia, p. 203–229. *In* W.J. Neil and D.C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State Unversity Press, Corvallis, Oreg.

Corporate author
American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C. 1193 p.

Theses
Kutty, M.N. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. Natl. Libr. Can., Can. Theses Microfilm No. 646. 102 p.

Reports
Smith, J.E. 1981. Catch and efforts statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation
Koike, A., and Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 11 p. (Translated from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h", "min," and "s" are not used, since they are symbols for hour, minute, and second in the sense of duration or the length of time. Thus, "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word list

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Recommendations aux auteurs

Journal canadien des halieutiques et aquatiques

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sousjacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles — études d'une portée générale, constituant des contributions inédites à la science.

Critiques — appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives — essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans un style simple.

Commentaires — opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur, *Journal canadien des sciences halieutiques et aquatiques*, Conseil national de recherches du Canada, Ottawa, ON K1A 0R6, Canada, **en triplicata** et accompagnés d'une lettre qui fait état (1) des principaux points et de l'importance du travail, (2) de la participation de tous les auteurs au manuscrit, de leur approbation de ce dernier et de l'assurance que le manuscrit en question n'est pas en cours d'examen aux fins de publications dans une autre revue, (3) de suggestions quant aux arbitres éventuels, (4) d'une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis et (5) des numéros de téléphone et de télécopieur ainsi que de l'adresse de courrier électronique de l'auteur correspondant. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les similis) jusquà ce qu'on les lui demande. **Afin de les protéger lors de l'expédition, les manuscrits et les illustrations doivent être solidement emballés.**

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Les auteurs sont vivement encouragés à soumettre, en plus de la copie imprimée, une copie sur disquette (3,5 ou 5,25 pouces) du manuscrit (version *acceptée* uniquement) préparée en logiciel de traitement de texte (accompagné d'une filière en format ASCII) sur ordinateur Macintosh our sur ordinateur personnel compatible à l'IBM. Le logiciel de traitement de texte et le numéro de version doivent être identifiés.

La publication d'un article fait foi du transfert volontaire, de la part de l'auteur, de tous les droits d'auteur au CNRC. Toute permission de reproduction ou de republication d'une partie ou de la totalité d'un article doit être obtenue auprès du Service de publication du CNRC.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814, U.S.A.) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103, U.S.A.). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (5e éd. 1991. Spec. Publ. No. 20, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le *Journal* suit les recommandations contenues dans Shaklee et al. (1990. Trans. Am. Fish. Soc. 119 : 2–15). Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, no 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association

canadienne de normalisation, 178, boul. Rexdale, Rexdale, ON M9W 1R3, Canada).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. **Il doit éviter de modifier le contenu du texte.**

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*. L'année 1995 a marqué l'adoption d'une mise en page refondue pour le *Journal*. Les auteurs voudront consulter une revue reflétant la nouvelle mise en page afin d'en connaître les détails de présentation, en particulier en ce qui a trait aux tableaux et aux bibliographies

Dactylographiez le manuscrit sur papier blanc (mesurant 8,5 sur 11 pouces) sur un côté de la page. Laissez toujours une marge d'au moins 1 pouce.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à **double interligne**. Si on ne dispose pas d'une police italique, souligner les passages à imprimer en italique.

En aucun endroit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il était plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé* donne-t-il l'essentiel des connaissances nouvelles ? L'*introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème? La section *matériel et méthodes* contient-elle le minimum de détails qui permettront aux lecteurs de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constatations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

(1) Limitez le résumé à un paragraphe d'environ 175 mots.

(2) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

(3) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

(4) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

(5) Dans les figures, les tableaux et le texte, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

(6) Évitez les formes ambiguës, telles que g $C/m^2/d$; utilisez plutôt g $C \cdot m^{-2} \cdot d^{-1}$.

(7) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire den donner la référence.

(8) Dans le texte, généralisez à partir des tableaux et des figures; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

(9) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

(10) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

(11) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

(12) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex. disponible auprès du Département d'économique, Université Simon Fraser, Burnaby, BC V5A 1S6, Canada.

(13) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1990) et ne soulignez pas *et al.*

(14) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

(15) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies ou des imprimés laser des figures, ou encore les originaux, s'ils ne dépassent pas 8,5 sur 11 pouces. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particu-

lièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : 1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et 2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que « Sur » ou « Vers », ou des expressions telles que « Contribution à...» ou « Recherche sur... ». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que « ...on y analyse » ou « ... a été trouvé »; soyez spéficique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail et qu'il soit complet en lui-même (c.-à-d. seule les références *essentielles* sont indiquées, sous forme retraçable, p. ex. R.B. Deriso. 1980. J. can. sci. halieut. aquat. 37 : 268–282).

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375–500 mots.

Matériel et méthodes

Cette section renforme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : « Afin de déterminer...nous... ». Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude; que ces réponses soient aussi complètes que possible, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendres devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. **On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa.** Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½ ou 2 colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des minuscules suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit « Tableau — près d'ici ».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies, des imprimés laser ou les dessins originaux (pourvu qu'ils ne dépassent pas 8,5 sur 11 pouces) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, 1½- ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elles devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge « Fig. — près d'ici ».

Les illustrations produites par imprimantes laser sont acceptables; les illustrations produites par imprimantes par points ne sont pas acceptables. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

Nous accordons une préférence au programme de graphiques CorelDraw!, grâce auquel nous pouvons traiter divers formats compatibles à l'IBM (p. ex. .EPS, .TIF). Le nom du programme, de la version, les numéros de figure, les noms de fichier ainsi que leur suffixe doivent être clairement indiqués sur toute disquette. Utilisez les suffixes normalisés pour les programmes utilisés. Le cas échéant, il se pourrait que nous ne puissions pas afficher ou ouvrir vos fichiers.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont débités à l'auteur; le prix actuel est de 1000 $ pour chaque page ou partie de page. Pour les reproductions en couleurs, ne soumettez que des photos d'excellente qualité. Nous préférons utiliser des photographies en couleurs, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs. Pour obtenir de plus amples renseignements, communiquez avec le Bureau de la rédaction ou le Bureau des pubilcations. On peut obtenir auprès du Bureau de la rédaction la brochure *Préparation des photographies et des illustrations en vue de la reprographie*, préparée spécialement par le Bureau des publications

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits ou souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Appendice

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimetrès par gramme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	dm
degré Celsius	°C
degrés de liberté	dl
écart type	ET
électronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J

jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	M
mole	mol
moles par litre	mol/L, M
pascal	Pa
seconde	s
semaine	sem
tonne (métrique)	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour-mois-année, p. ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont insérés dans le texte à un endroit approprié : « Brown (1983) compare... » ou « ...on a comparé (Brown 1983) ». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de « et al. » : « Brown et al. (1983) comparent... » ou « ...on a comparé (Brown et al. 1983) ».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de « communication personnelle ».

Données inédites

Si un livre ou un article inédit a été accepté pour publication, insérez-le dans la liste des références, avec la mention « (Sous presse) ». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si un article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention « données inédites ».

Liste de références

Les références doivent être classées par ordre alphabétique des noms des premiers auteurs. Si la liste comprend plusieurs publications d'un même premier auteur, les règles suivantes seront appliquées : (1) S'il n'y a qu'**un auteur**, les ouvrages se succèdent par ordre chronologique de parution en commençant par le plus ancien. Si l'auteur a publié, au cours d'une année, plusieurs livres ou articles, on distinguera les références les unes des autres en faisant suivre l'année de publication d'une lettre (*a*, *b*, *c*, etc.). (2) S'il y a **deux auteurs**, les ouvrages sont placés à la suite de ceux n'ayant qu'un auteur, et sont alors classés par ordre alphabétique du nom du deuxième auteur. (3) S'il y a **trois auteurs ou plus**, les ouvrages sont placés à la suite de ceux ayant deux auteurs et sont classés par ordre chronologique. La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après *CASSI* ou les *Serial Sources for the BIOSIS Data Base*).

Article du journal

Archambault, D., et E. Bourget. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctiques. Can. J. Fish. Aquat. Sci. 40 : 1278–1292.

Numéro complet du journal

Gordon, D.C., Jr., and A.S. Hourston (*Éditeurs*). 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1) : 365 p.

Livre dans une série

Hilton, J.W., et S.J. Slinger. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. Spéc. Can. Sci. Halieut. Aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

Forrester, W.D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans. Service hydrographique du Canada, Ottawa (Ont.).

Partie d'un livre

Aubert, M., J. Aubert, S. Daniel et N. Desirotte. 1982. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [ed.]. La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C. 1193 p.

Thèse

Delisle, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse doctorale, Université d'Ottawa, Ottawa (Ont.).

Rapports

Dutil, D.J., M. Fortin et Y. Vigneault. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. Manuscr. Can. Halieut. Aquat. 1653 : 29 p.

Traduction

Koike, A., et Ogura. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64 : 11 p. (Traduit du japonais par Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles « h, » « min » et « s » ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12 : 30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, « cold water » (n.) « cold-water » (adj.); « hard water » (n.), « hardwater » (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

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Canadian Journal of Zoology / Revue canadienne de zoologie	73	51	449	166	449	175	150
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Genome / Génome	38	70	262	108	262	116	75
Canadian Geotechnical Journal / Revue canadienne de géotechnique	32	53	226	88	226	95	50
Quarterly / Trimestrielle							
Environmental Reviews/ Dossiers environnement	3	244	163	57	163	63	25

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